

# **Blood Component Therapy in Trauma Haemorrhage**

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## Abstract

Exsanguination following severe injury remains the most common preventable cause of traumatic death. One third of these patients exhibit trauma-induced coagulopathy (TIC) with an associated significant morbidity and mortality. A key feature of damage control resuscitation (DCR) is early diagnosis and direct targeting of TIC with blood component therapy combined in major haemorrhage protocols (MHPs). The impact and efficacy of high-dose blood component therapy on TIC is currently unknown. The overall aim of this thesis is to address these specific areas of uncertainty.

A prospective observational cohort study of 106 severely injured, bleeding trauma patients was performed over a three-year period. Blood samples for coagulation testing and clotting factor analysis were drawn on arrival and during the acute bleeding (resuscitative) phase after administration of every 4 U of PRBCs, up to 12 U. The quantity of blood products administered within each interval was recorded.

Following implementation of MHP significantly higher ratios of blood component therapy were observed. FFP:PRBC transfusion improved from 1:3 to 1:2 ( $p<0.01$ ) and CRYO:PRBCs from 1:10 to 1:7 ( $p<0.05$ ). There was a six-fold reduction in platelets wastage (14% to 2%,  $p<0.01$ ). On admission, 43% of patients were coagulopathic and increased to 49% by PRBC 4, 62% by PRBC 8 and 68% by PRBC 12, despite adherence to DCR strategies. In shock, lactate clearance did not occur until haemorrhage control was achieved with no further PRBC requirement. Only the combination of high-dose FFP, CRYO *and* platelet therapy with a high total fibrinogen load produced a consistent improvement in ROTEM parameters.

The body of work within this thesis supports the need for larger studies to determine the clinical benefits of early fibrinogen supplementation in treating severely injured trauma patients suffering from life threatening haemorrhage.



## Declaration

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## **Publications**

Davenport R, **Khan S**. Management of major trauma haemorrhage: treatment priorities and controversies. Br J Haematol. 2011 Dec; 155(5): 537–48.

**Khan S**, Allard S, Weaver A, Barber C, Davenport R, Brohi K. A major haemorrhage protocol improves the delivery of blood component therapy and reduces waste in trauma massive transfusion. Injury. 2013 May; 44(5): 587–92.

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## List of Abbreviations

<b>ACIT II</b>	<b>activation of coagulation and inflammation in trauma II</b>
<b>AIS</b>	<b>abbreviated injury scale</b>
<b>ANOVA</b>	<b>analysis of variance</b>
<b>aPTT</b>	<b>activated partial thromboplastin time</b>
<b>ARDS</b>	<b>acute respiratory distress syndrome</b>
<b>ATC</b>	<b>acute traumatic coagulopathy</b>
<b>ATLS</b>	<b>advanced trauma and life support</b>
<b>BP</b>	<b>blood pressure</b>
<b>BD</b>	<b>base deficit</b>
<b>Ca<sup>2+</sup></b>	<b>ionised calcium</b>
<b>cc/kg</b>	<b>cubic centimetres per kilogram</b>
<b>CI</b>	<b>confidence interval</b>
<b>cm</b>	<b>centimetre</b>
<b>CO<sub>2</sub></b>	<b>carbon dioxide</b>
<b>CRYO</b>	<b>cryoprecipitate</b>
<b>DALY</b>	<b>disability adjusted life years</b>
<b>DIC</b>	<b>disseminated intravascular coagulopathy</b>
<b>DCR</b>	<b>damage control resuscitation</b>
<b>DCS</b>	<b>damage control surgery</b>
<b>ED</b>	<b>emergency department</b>
<b>ELISA</b>	<b>enzyme-linked immunosorbent assay</b>
<b>FBC</b>	<b>full blood count</b>
<b>FFP</b>	<b>fresh frozen plasma</b>
<b>FWB</b>	<b>full whole blood</b>
<b>g</b>	<b>gram</b>
<b>g/dl</b>	<b>gram per decilitre</b>
<b>g/l</b>	<b>gram per litre</b>
<b>GCS</b>	<b>Glasgow coma score</b>
<b>GPIb</b>	<b>glycoprotein 1b</b>
<b>h</b>	<b>hours</b>
<b>hr</b>	<b>heart rate</b>
<b>HR</b>	<b>haemostatic resuscitation</b>
<b>ICU</b>	<b>intensive care unit</b>
<b>INR</b>	<b>international normalised ratio</b>

<b>IQR</b>	<b>interquartile range</b>
<b>ISI</b>	<b>international sensitivity index</b>
<b>ISS</b>	<b>injury severity score</b>
<b>i.v.</b>	<b>intravenous</b>
<b>kg</b>	<b>kilogram</b>
<b>kPa</b>	<b>kilo Pascal</b>
<b>l</b>	<b>litres</b>
<b>M</b>	<b>molar</b>
<b>MABP</b>	<b>mean arterial blood pressure</b>
<b>mEq/l</b>	<b>molar equivalent per litre</b>
<b>mg</b>	<b>milligram</b>
<b>MHP</b>	<b>major haemorrhage protocol</b>
<b>min</b>	<b>minute</b>
<b>ml</b>	<b>millilitre</b>
<b>mm</b>	<b>millimetre</b>
<b>mmHg</b>	<b>millimetres mercury</b>
<b>MOF</b>	<b>multiple organ failure</b>
<b>mol/l</b>	<b>mole per litre</b>
<b>mmol/l</b>	<b>millimole per litre</b>
<b>MT</b>	<b>massive transfusion</b>
<b>MTP</b>	<b>massive transfusion protocol</b>
<b>NaCl</b>	<b>sodium chloride</b>
<b>n</b>	<b>number</b>
<b>NAO</b>	<b>national audit office</b>
<b>NCEPOD</b>	<b>national confidential enquiry into patient outcome and death</b>
<b>NIH</b>	<b>national institute of health</b>
<b>O<sub>2</sub></b>	<b>oxygen</b>
<b>OD</b>	<b>oxygen debt</b>
<b>OR</b>	<b>odds ratio</b>
<b>PL</b>	<b>phospholipid</b>
<b>PLT</b>	<b>platelet</b>
<b>PRBCs</b>	<b>packed red blood cells</b>
<b>PT</b>	<b>prothrombin time</b>
<b>PeLAR</b>	<b>personal, legally appointed representative</b>
<b>PrLAR</b>	<b>professional, legally appointed representative</b>
<b>PTSD</b>	<b>post-traumatic stress disorder</b>
<b>PTr</b>	<b>prothrombin time ratio</b>

<b>RLH</b>	<b>Royal London Hospital</b>
<b>rpm</b>	<b>revolutions per minute</b>
<b>rr</b>	<b>respiratory rate</b>
<b>RR</b>	<b>relative risk</b>
<b>RTC</b>	<b>road traffic collision</b>
<b>s</b>	<b>second</b>
<b>SBP</b>	<b>systolic blood pressure</b>
<b>TARN</b>	<b>the trauma audit and research network</b>
<b>TCAU</b>	<b>trauma clinical academic unit</b>
<b>TF</b>	<b>tissue factor</b>
<b>TIC</b>	<b>trauma-induced coagulopathy</b>
<b>TNF<math>\alpha</math></b>	<b>tumour necrosis factor alpha</b>
<b>tPA</b>	<b>tissue type plasminogen activator</b>
<b>TRALI</b>	<b>transfusion-related acute lung injury</b>
<b>TTL</b>	<b>trauma team leader</b>
<b>TXA<sub>2</sub></b>	<b>thromboxane A<sub>2</sub></b>
<b>U&amp;E</b>	<b>urea and electrolytes</b>
<b>vWF</b>	<b>Von Willebrand factor</b>
<b>°C</b>	<b>degrees Celsius</b>
<b><math>\mu</math></b>	<b>micro</b>
<b><math>\chi^2</math></b>	<b>Chi-squared test</b>

## CHAPTER ONE

# **Introduction**

## 1.1 The significance of trauma

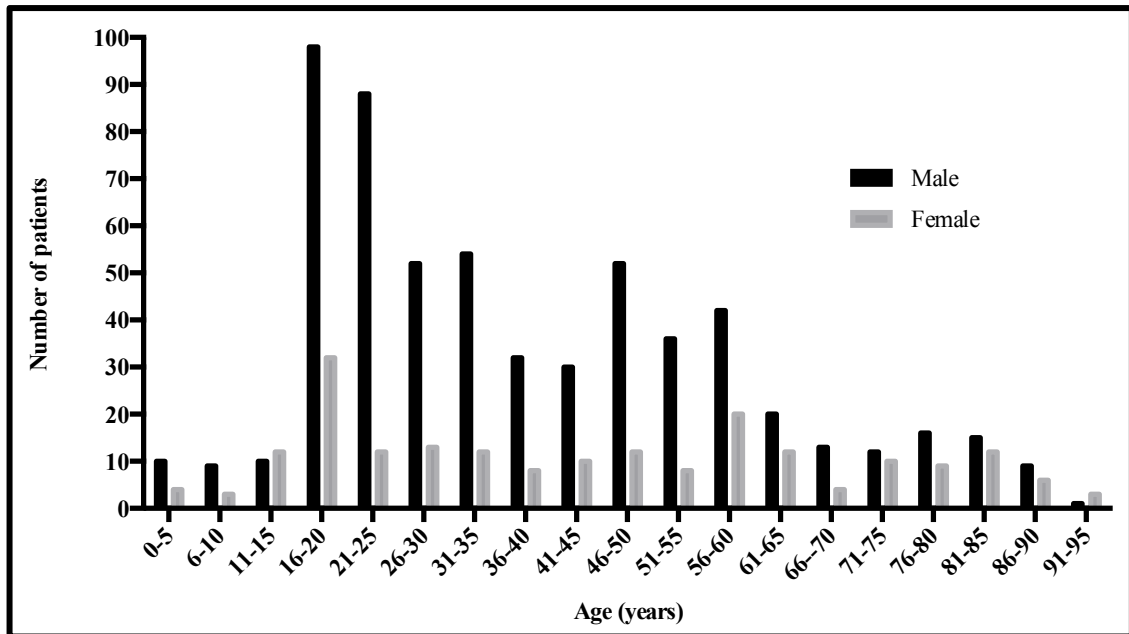
The body of work presented in this thesis reports the management of severe, and often life-threatening, haemorrhage in trauma.

The definition of trauma encompasses any injury resulting from penetrating or blunt physical force such as stabbings, falls or road traffic accidents (RTCs). It is undoubtedly one of the major healthcare issues faced by modern society, resulting in the annual death of more than five million people worldwide; this number is expected to increase to more than eight million by 2020 (1). In 2013 in the US, injuries accounted for 59% of all deaths among persons from one to 44 years of age, which is more deaths than non-communicable diseases and infectious diseases combined (2).

In the UK, traumatic injury accounts for over 17,000 deaths annually (3). Approximately half of the patients who die from severe injuries do so prior to reaching hospital, either before the arrival of pre-hospital medical services, or due to rapid deterioration despite initial resuscitative attempts to stabilise them.

Trauma is a disease and it is a condition that predominantly affects young adults. It kills more people between the ages of 15 and 44 than any other disease (*Figure 1.1*), with an average of 36 life years lost per trauma death (4). Not only does traumatic injury carry a high mortality rate, but it also is estimated that for every death due to injury, there are two patients left with permanent serious disabilities (5). In 2004, a report from the World Health Organisation stated that the average global burden of disease was 237 DALYs per 1,000 population (6). RTCs alone were the ninth leading cause, accounting for 2.7% of the total. Comparatively, the principal source of disease burden was lower respiratory tract infections, which constituted only a slightly higher 6.2% of all DALYs (6). In addition to

the personal and emotional effects, trauma inflicts a massive financial burden in terms of direct healthcare costs, as well as loss of productivity to society.



**Figure 1.1. Civilian trauma patients by age and gender.** A 2007 UK study of 795 major trauma patients carried out by the NCEPOD found that 75% of major trauma patients were male, with a high concentration of those between the ages of 16 to 20 (5).

The severity of the health impact associated with trauma is multifactorial. Following the initial injury, the trauma patient may not only be affected with severe physical impairments but may also suffer from equally disabling psychological disorders such as PTSD (7). Injured individuals may experience dramatic changes in their social circumstances, with an inability to return to work, thereby incurring financial hardship not only for them but also for their dependents. Others may have problems integrating back into society, either from a physical or a cognitive impairment. These damaging aftermaths of injury are not solely limited to the trauma patient but are far reaching. Family and friends may become

permanent caregivers for those with physical and cognitive disabilities and may have to dramatically alter their own lifestyles.

## 1.2 Classification and characteristics of trauma

Trauma is classified using an injury severity score (ISS) (8). The ISS is an anatomical scoring system that retrospectively assigns a measure of severity ranging from zero to 75, with a score of 16 or greater signifying major trauma. To calculate ISS, each injury is initially assigned an abbreviated injury score (AIS). The AIS is an anatomical scoring system first introduced in 1976 (9). It has subsequently undergone numerous revisions and has been updated against survival so that it now provides accurate ranking of the severity of injury. Injuries are ranked on a scale of 1 to 6, with 1 being minor, 5 severe, and 6 representing a non-survivable injury (*Figure 1.2*). However, AIS represents the ‘threat to life’ associated with an injury and is not meant to represent a comprehensive measure of severity.

Injury	AIS Score
1	Minor
2	Moderate
3	Serious
4	Severe
5	Critical
6	Non-survivable

**Figure 1.2. AIS scoring system.** (*Adapted from Copes WS, Sacco WJ, Champion HR, Bain LW, “Progress in Characterising Anatomic Injury”. In Proceedings of the 33rd Annual Meeting of the Association for the Advancement of Automotive Medicine, Baltimore, MA, US, 205–218.*)



To calculate ISS, the AIS from the three most severely injured body regions are squared and added together. Mortality increases with ISS (*Table 1.1*), and a score of 75 signifies injuries that are unlikely to be survived (5).

ISS	Percentage of major trauma patients	Percentage mortality of this injury severity score group
16–25	62.6	10.5
26–40	28.9	22.1
41–74	7.7	44.3
75	0.8	76.6

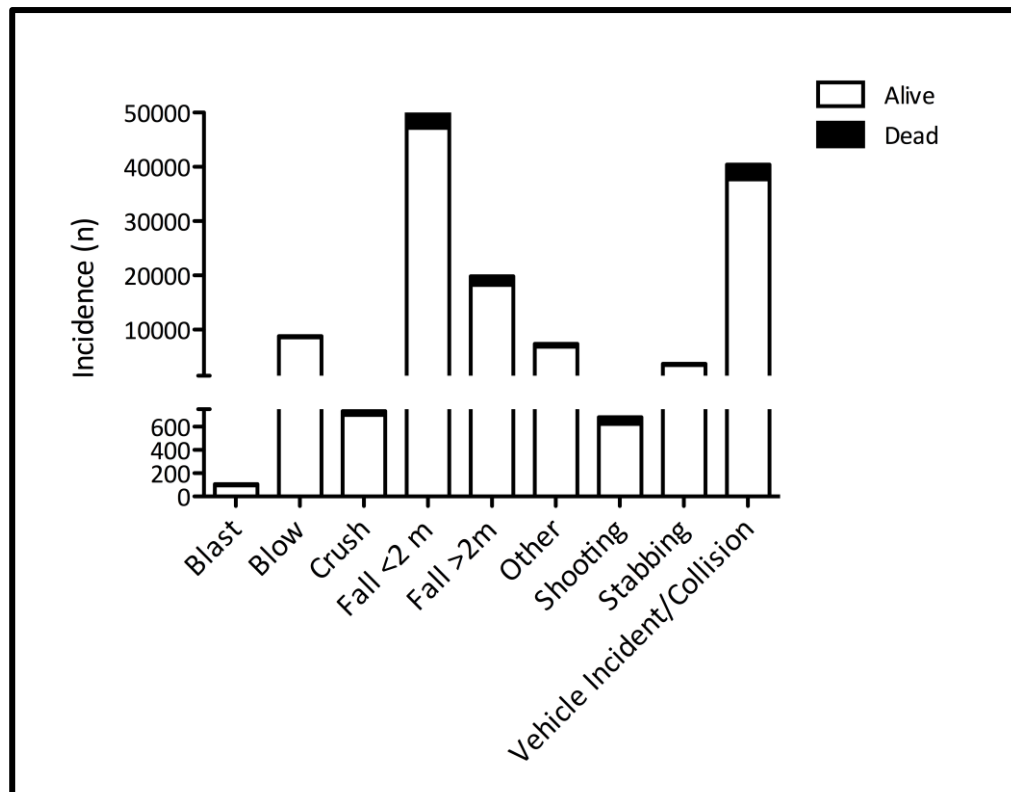
**Table 1.1. ISS group and mortality.** (*Data courtesy of TARN (5).*)

The trauma population itself can be further broadly divided into either military or civilian cohorts that exhibit both comparable and contrasting characteristics. In the military setting, casualties are almost exclusively young males in their 20s. Data that has been collected from Operation Iraqi Freedom (10) reported that over 80% of those that were killed or injured were a direct result of penetrating missile explosions. The origin of such a missile (e.g. explosion, gunshot) depends largely upon the nature of the battle, enemy tactics and the geographical location of the combat theatre. When examining civilian trauma, while there is also a higher prevalence of male subjects, the gender ratio is rather more balanced and the average age of victims tend to be higher than that of the military population.

### 1.3 Mechanism of injury

Among the UK civilian population, a blunt mechanism of injury predominates, with fall from a height and RTCs representing the most common causes (11, 12). Naturally, specific characteristics vary between each country. In the UK itself, 98% of trauma is caused by blunt force and the most common mechanisms of injury are falls and RTCs. Major blunt

trauma can also be caused by assault, burns, blasts, crushes and self-inflicted injuries, such as hanging. Penetrating injuries such as knife or gunshot wounds and impalements account for only 2% of major trauma, but this figure is predicted to increase (13) (*Figure 1.3*).



**Figure 1.3.** A summary of trauma in England and Wales from 2002 to 2011. The category 'Blow' includes blunt assaults, while 'Others' relate to injuries sustained following industrial and farming accidents, for example. (Data courtesy of the Trauma Audit and Research Network.)

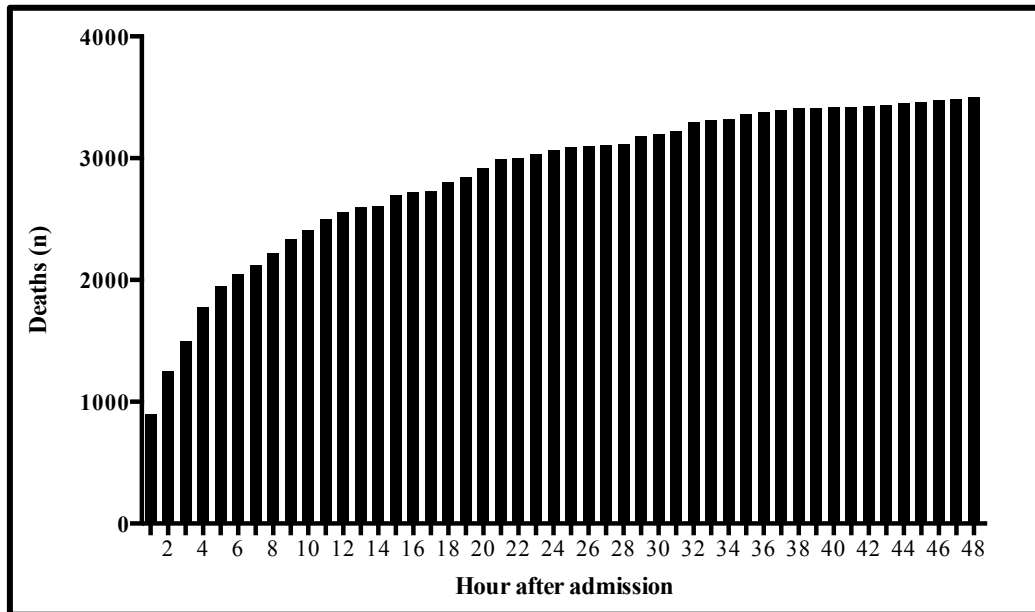
## 1.4 Mortality following trauma

Regardless of the mechanism of injury, the temporal distribution and modes of mortality in both cohorts bear similarities. Historically, there has been a trimodal description of trauma deaths (14). The first peak is immediate deaths that occur within one hour of the injury. About 50–70% of all trauma deaths are said to belong to this group; however, the data vary based on various factors, such as type of injury, region, or type of emergency medical

system. It is reported that up to 60% of trauma deaths occur immediately after injury or before hospital arrival (11, 15, 16). The majority of these patients die due to exsanguination (massive blood loss), from major vessel disruption or a catastrophic head injury and, for the most part, are unresponsive to any current medical intervention.

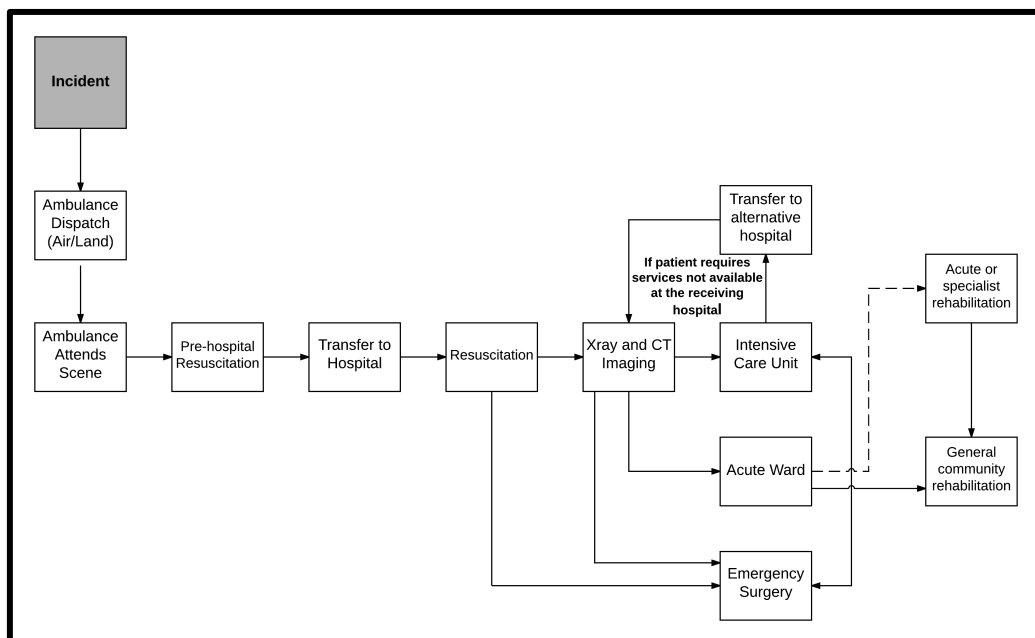
The second distribution is within one to four hours after the injury and the third distribution around one to two weeks later. Datasets from two large European trauma registries showed that about 50% of all in-hospital trauma deaths occur within the first 24 h after admission ((17, 18) *Figure 1.4*) and, within those initial 24 h, in excess of 70% deaths occur in the first six h after admission. These represent the greatest potential for salvage but are also the most difficult group of trauma patients to investigate (19, 20).

The mode of death of these patients is divided equally between central nervous system injury and severe haemorrhage. Lefering et al., concluded that injury severity and massive haemorrhage were the most prevalent among very early hospital deaths (up to six h after admission to the ED), while the highest rates of severe head injury were found in patients who died in the first week (17, 18). Late deaths are frequently associated with complications such as organ failure or sepsis and increasing age (17, 18). With timely diagnosis and intervention, haemorrhage is reversible and is the leading cause of preventable death after trauma. Studies from the military and civilian community suggest that up to one third of haemorrhage-related deaths could be avoided by expedient control of bleeding (21-23).



**Figure 1.4. Cumulative number of non-survivors for the first 48 h after hospital admission.** Only patients from the Trauma Audit and Research Network (TARN) database with available time of admission and time of death are considered ( $n = 3,584$ ) (Modified from Lefering et al., 2012 (18))

The overview for the current pathway for major trauma in the UK is detailed below in Figure 1.5.



**Figure 1.5. The current patient pathway for major trauma in the UK.** (Ordered by the House of Commons, Report by the Controller and Auditor General, 2010 (24).)

## 1.5 Trauma and research

Traumatic injuries are a public health problem of vast proportions. In the past, the National Academy of Sciences characterised accidental death and disability as the “neglected disease of modern society” (25). Yet, in spite of the global impact of trauma, it remains a field characterised by the lack of evidence-based practice. This lack of knowledge is due to a relative paucity of research. The RAND report in the UK (24) and the National Trauma Institute in the US (26) have both recently highlighted that trauma remains a poorly recognised public health problem with disproportionately low investments in research. Despite repeated reports identifying poor trauma practice, the Department of Health and the NHS have taken little action to improve major trauma care (13). Furthermore, deficiencies in major trauma care were identified by the Royal College of Surgeons as early as 1988, but there has been little progress since. In 2007, a report by the NCEPOD concluded that 60% of major trauma patients received a standard of care that was ‘less than good practice’ (27). A number of reports, including one by the National Audit Office (NAO) in 1992, have made recommendations about the information and actions required to improve the delivery of major trauma care, but there remains significant data gaps and a lack of formalised systems.

In December 2010, the UK Department of Health (DH) requested RAND Europe to provide a strategic, rapid review of the funding landscape for complex trauma research in the UK. In this review, they were asked to focus on those areas of research that target the early phase of injury and can improve the effectiveness of therapies and interventions at the pre-hospital and early in-hospital phase. The RAND report concluded that “complex trauma is an orphan and niche area of research that is disproportionately resourced in comparison to the burden of the disease” (24). However, less than 1% of the total UK public expenditure on health research is allotted to trauma research. In the US, in terms of potential life years

lost (defined by the millions of dollars per years of potential life lost per 100,000 population), the National Trauma Institute (NTI) support ratio for trauma is only 10 cents, as compared to the US \$3.51 for HIV and US \$1.65 for cancer (26) .

There are other specific challenges associated within trauma research besides poor funding. Trauma remains a challenging field for clinical research given the difficulty of enrolling patients who may present in physiological extremis, and outside traditional working hours. This is further compounded by the problems associated in obtaining consent in patients who may be unconscious on arrival to the hospital, distressed or lacking capacity. Consequently, not only is the overall volume of research in trauma limited, but the specific clinical fields investigated within the specialty are narrow.

## **1.6 The cost of major trauma**

The cost of treating major trauma within the UK is not known with any real clarity. It is estimated, however, that major trauma costs the NHS a minimum of somewhere in the region of £0.3 billion and £0.4 billion a year. This estimate is calculated on the basis of the average costs of treating blunt and penetrating trauma, collected through academic research (28), and the estimate of the number of cases of major trauma. It includes the cost of ambulance transportation, the immediate hospital stay, and the cost of all procedures performed during the entire hospital admission. The costs of long-term hospital treatments, rehabilitation, home-care support, or informal carer costs after discharge remain unknown, but research suggests that, for major trauma, the majority of the costs are incurred following the initial period of hospitalisation. Furthermore, the NAO estimates that the annual lost economic output as a result of major trauma is between £3.3 billion and £3.7 billion (13).

## 1.7 Trauma and haemorrhage

Uncontrolled haemorrhage or exsanguination is responsible for over 40% of early in-hospital trauma mortality (19) and is a leading cause of preventable death (29, 30). Furthermore, massive haemorrhage in most trauma patients is multifactorial and presents as a combination of both diffuse bleeding as a result of coagulopathy and bleeding from the vasculature that needs to be addressed surgically. Even though surgical bleeding can, in most instances, be controlled by the intervention of a trauma surgeon, control of coagulopathic bleeding is often difficult, and sometimes impossible (31). Moreover, it is estimated that 25–33% of severely injured, bleeding patients arrive at hospital with significant derangements in blood coagulation (32). It is this specific subset of patients that represent potentially reversible deaths if identified and treated early and aggressively (33).

This derangement in coagulation function is known as the Acute Traumatic Coagulopathy (ATC) and patients presenting with this phenomenon have a mortality rate approaching 50%. They have significantly greater transfusion requirements, organ injury, septic complications and critical care stay (34) (35) (36). Furthermore, transfusion of allogeneic blood replacement products during resuscitation inflicts a dose-dependent increase in the extent of these outcomes independent of shock severity (37). It is for this paramount reason that minimising blood loss is an absolute clinical priority after traumatic injury. Specific management strategies targeting ATC may allow significant improvement in outcomes (38) (39, 40) (41). These “damage control” resuscitation (DCR) (39) approaches require early identification of ATC to allow rapid activation of major haemorrhage protocols (MHPs).

However, prediction of ATC from admission clinical parameters is unreliable, and traditional laboratory-based clotting tests have logistical issues that limit their utility in the acute trauma setting (42–44). In the absence of a diagnostic tool, current ATC management relies on empiric transfusion strategies activated on the basis of clinical surrogates of

haemorrhage or a physician's "best guess" approach (45) (46) (23, 39). This results in delayed correction of ATC and is associated with suboptimal blood product usage (47) (48). Inadequate or sub-therapeutic transfusion has been proven to be associated with poor outcomes (46), but over-transfusion of blood components may result in dangerous additional donor exposures, such as Hepatitis and HIV, and furthermore is wasteful of a precious resource. In addition, PRBCs and other blood products are independently associated with reduced survival, excess morbidity, sepsis, and organ failure (49-51). Moreover, targeted transfusion therapy for severe traumatic haemorrhage based on comprehensive and rapid measures of coagulation may lead to improved outcomes while optimising blood utilisation, but there remains a lack of high-level evidence to support this (52-56).

## **1.8 Trauma-induced coagulopathy**

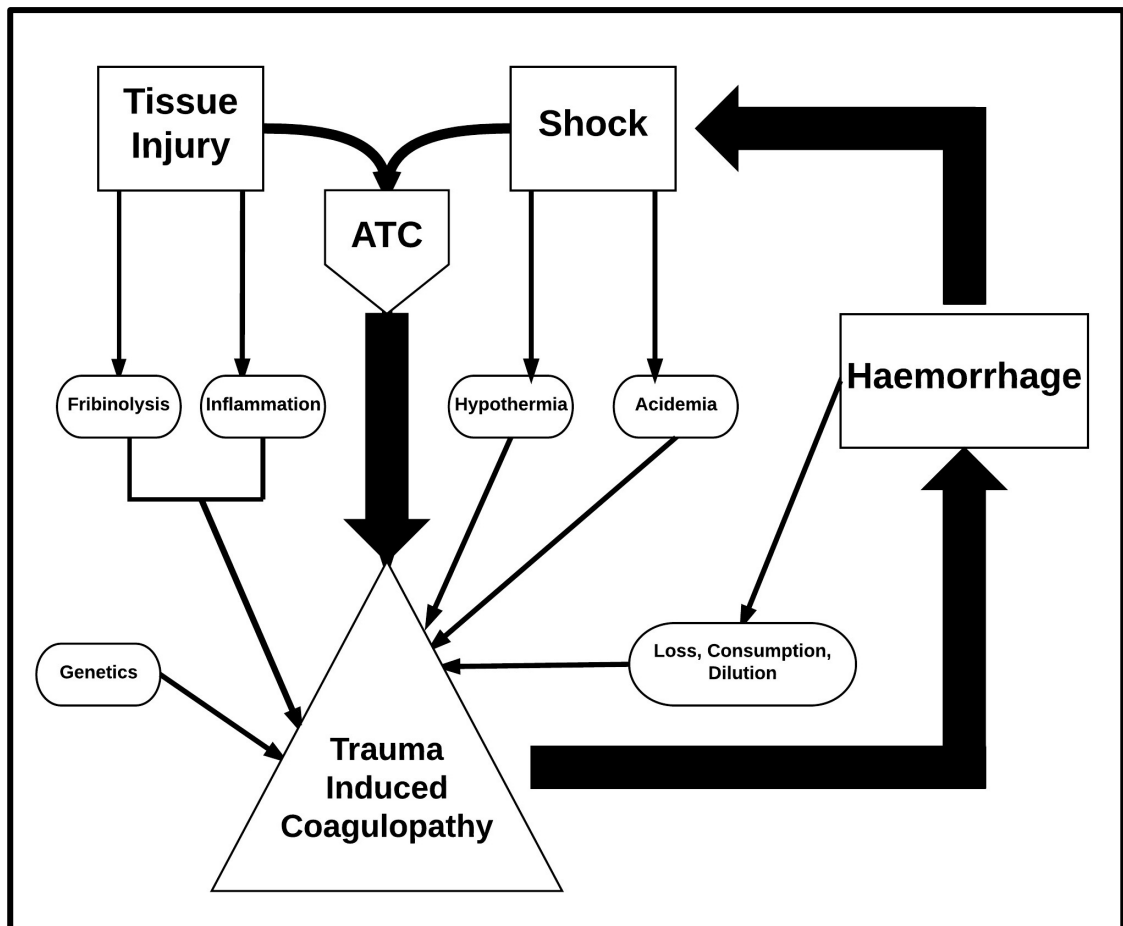
Coagulopathy is a disease or condition affecting the blood's ability to form a clot (57). Major injury and uncontrolled blood loss is often associated with trauma-induced coagulopathy (TIC) (*Figure 1.6*). Therefore, the most acute threat to an exsanguinating trauma patient is a bleeding diathesis. Impairments of normal haemostatic function after trauma results in difficult haemorrhage control, increased transfusion requirements and increased mortality (58).

Different factors influence the severity of TIC. On the one hand, the coagulopathy can be influenced by environmental and therapeutic factors that may contribute, in part, to acidaemia, hypothermia, dilution, hypoperfusion and coagulation factor consumption (59). On the other hand, TIC can also be affected by individual patient-related factors, including genetic background, co-morbidities, inflammation and medications, especially anticoagulants, and pre-hospital fluid administration (59).



TIC has been recognised for decades and historically described in the literature as a sequelae of the “lethal triad” of **coagulopathy**, **hypothermia** and **acidosis** and associated dysfunction or consumption of coagulation proteases (60). Historically, loss of coagulation factors is explained by haemorrhage (loss) or consumption, dilution from crystalloid or colloid administration and massive whole blood transfusion. Abnormality in the function of proteases was thought to be a cause of hypothermia and the effect of acidosis on enzyme function. However, in severely injured trauma patients, such variables frequently occur in tandem; hence, determining the independent detrimental effect of each is often difficult.

Clinically, TIC has many drivers and involves all aspects of haemostasis. The principle driver of TIC was previously considered to be haemodilution, but this is now thought to be an unlikely cause for the early coagulopathy seen following trauma, especially in the context of permissive hypotension and restrictive volume resuscitation. Furthermore, there is only weak evidence to suggest that direct tissue trauma in isolation consumes clotting factors akin to DIC, and there appears to be a decrease in fibrinogen utilisation during early coagulopathy (61). Coagulation is initiated by tissue damage (direct trauma) following exposure of procoagulant substances in the endothelium but coagulopathy is rare in severely injured patients without concomitant profound tissue hypoperfusion (shock) (61).

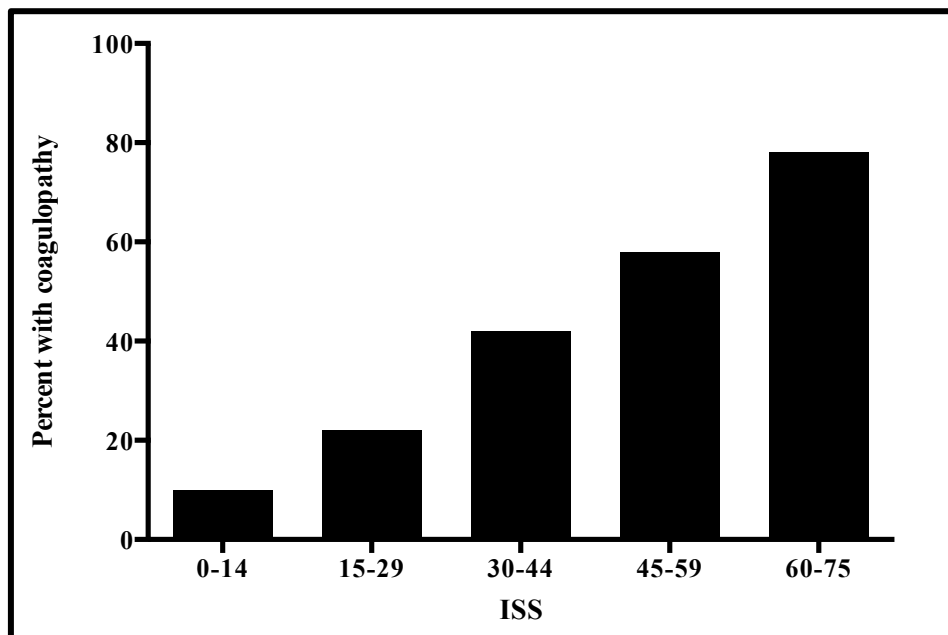


**Figure 1.6. The multifactorial drivers of TIC, showing some of the mechanisms that give rise to TIC. (Adapted from Davenport, Khan 2011 (62).)**

## 1.9 Acute traumatic coagulopathy

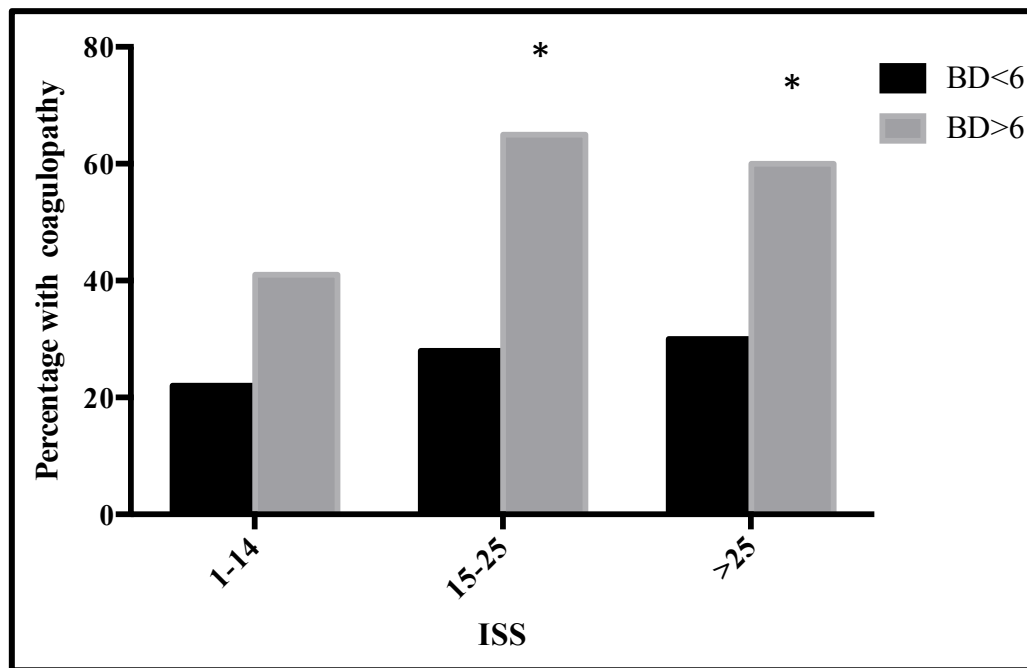
The landmark report of the existence of ATC was from a retrospective study of the admission coagulation results of 1,088 trauma patients transferred to the Royal London Hospital (RLH) by air ambulance (63). More recent studies have shown that shock and tissue trauma appear to act as primary drivers of coagulopathy in the immediate phase following injury (32) (64, 65) (66). ATC has been identified in up to 25% of trauma patients on admission to the hospital ED (35) (34, 67) (68). Trauma patients that were diagnosed with ATC on admission were observed to have a four-fold increase in mortality, greater transfusion requirements, required longer time within the intensive care unit (ICU), and face a significantly higher incidence of sepsis, organ injury and MOF (68).

These initial studies showed that ATC is closely correlated with the severity of tissue trauma (34, 36) and tissue hypoperfusion (67). Brohi et al. showed that, as the level of tissue trauma increased (rising ISS), the incidence of coagulopathy increased, such that nearly two thirds of patients with an ISS greater than 45 arrive with significant derangement of their haemostatic mechanisms. These authors also reported that trauma patients with coagulopathy were more likely to die than those without for any given ISS. Furthermore, trauma patients with coagulation abnormalities are known to develop organ dysfunction and spend longer in the critical care unit (69). Thus, injury severity is closely associated with the degree of acute coagulopathy seen after trauma. This London study reported only 10.8% of patients with an ISS of 15 or below had a coagulopathy, compared with 33.1% of those with an ISS over 15 (63) (*Figure 1.7*). This figure increased to 61.7% for those with an ISS over 45. However, tissue injury on its own is not sufficient to cause ATC. Shock with tissue hypoperfusion is a strong independent risk factor for poor outcomes in trauma (70, 71) and has been implicated in the pathogenesis of ATC (72).



**Figure 1.7. Incidence of coagulopathy.** (*Adapted from (63).*)

In these studies, resuscitation with crystalloid fluid was minimal and no patients were sufficiently hypothermic ( $<33^{\circ}\text{C}$ ) to impair coagulation protease activity, thrombin generation or reduce platelet function (73) (74). Hence, an alternative mechanism responsible for early coagulopathy was suggested. ATC is characterised by systemic anticoagulation and hyperfibrinolysis through endothelial activation of Protein C (62, 67, 75). Uncontrolled exsanguination with associated physiological disturbances (e.g. hypothermia and acidosis complicated by the dilutional effects of intravenous fluid resuscitation) exacerbate ATC and give rise to TIC (*Figure 1.6*). Previous work from our group on the study of acute coagulopathy found that no patient with a normal BD had prolonged PT or aPTT, regardless of injury severity or the amount of thrombin generated (76). In contrast, there was a dose-dependent prolongation of clotting times with increasing systemic hypoperfusion. Only 2% of patients with a BD under six mmol/l had prolonged clotting times, compared with 20% of patients with a BD over six mmol/l. Another study of 391 combat casualties in the Iraq War found that, for a given injury severity, patients who were shocked had a higher prevalence of ATC (77) (*Figure 1.8*). Nonetheless, the precise effects of acidaemia on coagulation remain unknown as it is clinically difficult to ascertain the precise inhibitory effects of pH vs tissue hypoperfusion and shock.



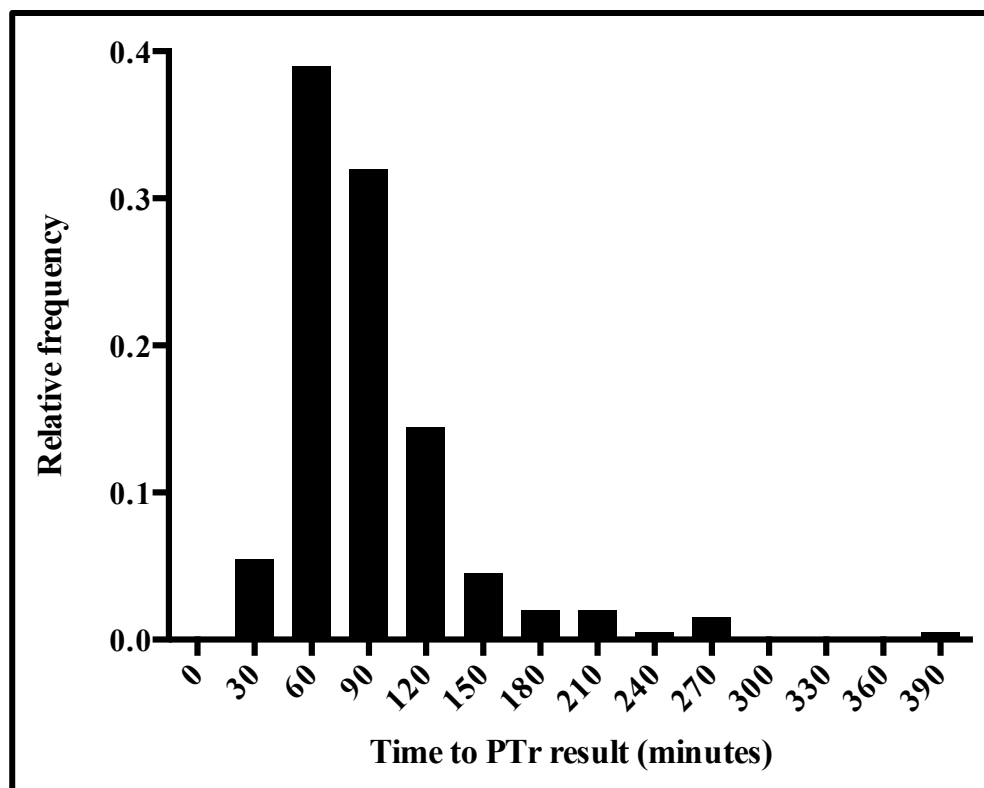
**Figure 1.8. Prevalence of coagulopathy by ISS and BD in patients requiring a blood transfusion.** (Adapted from \* $p < 0.05$  (78).)

## 1.10 Coagulation tests for the diagnosis of ATC

A knowledge gap exists with regard to the most appropriate tests for coagulation in trauma as there are no widely used validated and rapidly available investigations that can reliably guide transfusion therapy in an actively bleeding trauma patient (42, 79). Quantitatively measuring excess blood loss attributable to clinical coagulopathy is fraught with difficulty. Historic coagulation screening tests such as PT, aPTT, platelet count and fibrinogen levels are of limited value in acute exsanguination.

The PT and aPTT are screening tests that measure the ‘extrinsic’ and ‘intrinsic’ pathways of coagulation respectively. They report the time taken for initial fibrin polymerisation of platelet poor plasma, at 37°C, in response to exogenous stimulation of coagulation. As such, they neglect the pivotal role of platelets, do not measure clot strength, and may not reflect haemostasis *in vivo* (42, 79). Laboratory-based assays in platelet-poor plasma (PT,

aPTT, fibrinogen) require standard processing with results that are often not available to the treating clinician for upwards of 30 minutes (*Figure 1.9*) (42). As previously mentioned, PT and aPTT provide only partial information on clot initiation (80). These tests were originally designed for monitoring patients prescribed oral anticoagulants or suffering from congenital defects of haemostasis. Their widespread use in medicine means clinicians are familiar with them, and they have become the most frequently used method for assessing coagulation status in a vast number of medical disciplines, including trauma care.

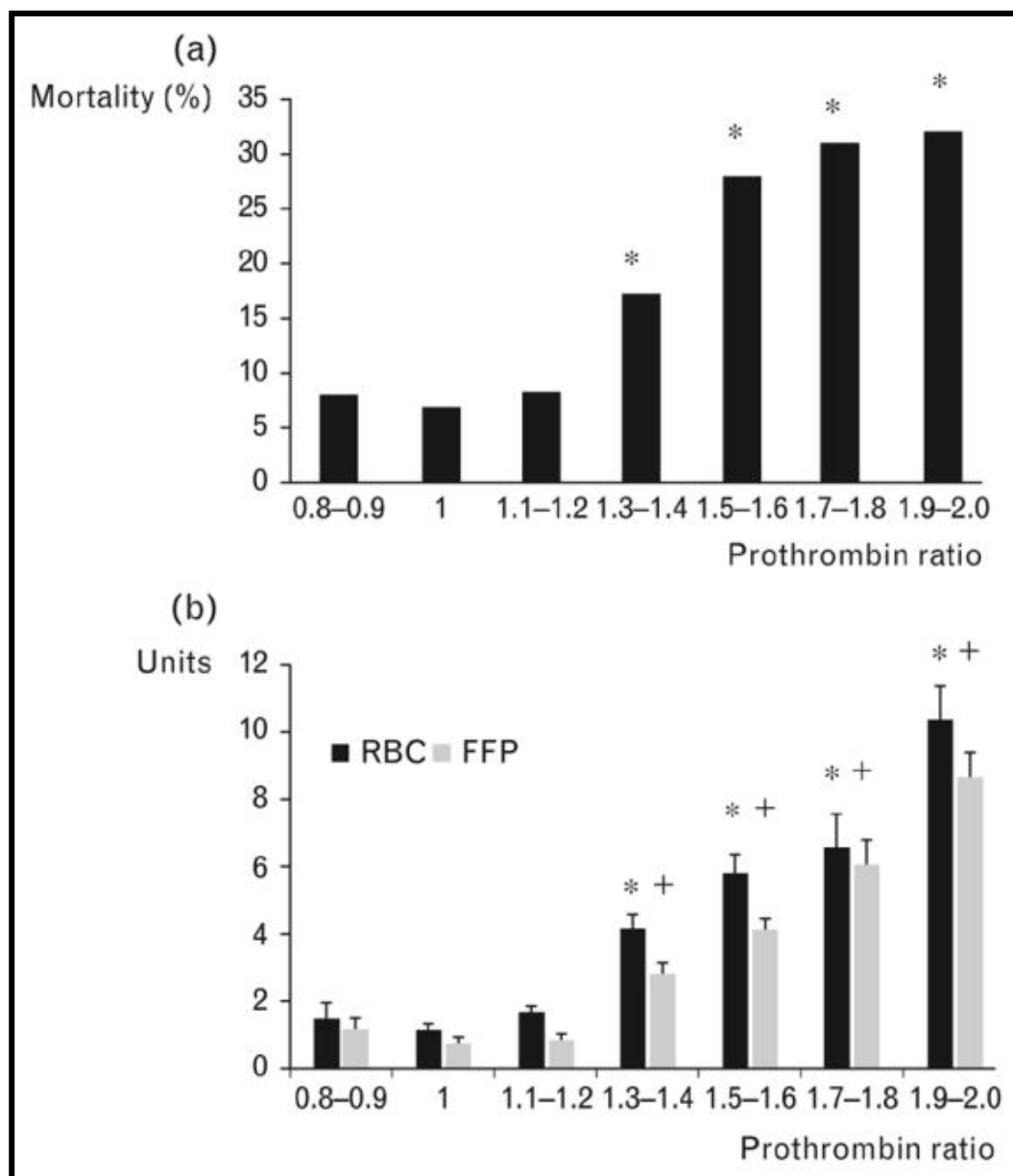


**Figure 1.9. Relative time to PT result.**

A platelet count and fibrinogen concentration assay usually supplement routine tests of coagulation. However, platelet counts are often normal in trauma patients and fail to provide any measure of platelet dysfunction secondary to the physiologic derangement evident in TIC (32). Furthermore, using the platelet count to guide therapy is difficult due to the lack of evidence for the clinical relevance of abnormal results. The Clauss fibrinogen assay is a non-functional test and is unable to assess the cross-linking of polymerised fibrin

by factor XIII. Moreover, none of the assays are able to evaluate the rate of clot propagation vs clot lysis or overall clot strength. In summary, the limitations of these tests are: they are not available within useful timeframes and they are unable to measure the full pattern of clotting dysfunction and therefore cannot effectively guide treatment in patients suffering from massive haemorrhage.

In modern-day practice, the vast majority of guidelines for diagnosis and management of major haemorrhage are based on arbitrary laboratory triggers, e.g.  $PT > 1.5 \times \text{normal}$  (81) (82). More recent work from our group showed that a PTr of 1.2 is a clinically significant threshold for the definition of ATC (65). A  $PTr > 1.2$  was associated with a stepwise increase in mortality and blood product requirements, and the group was able to demonstrate that the often-cited threshold for coagulopathy of  $PTr/IN > 1.5$  failed to detect 16% of patients with worse outcomes (*Figure 1.10*). Blood loss in severely injured trauma patients occurs at an alarming rate and these coagulation screens have questionable diagnostic sensitivity (83) and predictive power (84) to guide massive transfusions (MT). The “gold standard” test of coagulation in trauma should be robust in an emergency environment and should be able to rapidly evaluate all stages of whole blood clot formation and breakdown, while detailing the contribution of platelets and fibrinogen to clot function. One such diagnostic tool is rotational thromboelastometry (ROTEM).



**Figure 1.10. Relationships between ATC and clinical outcomes.** (A) Increasing mortality with increasing prolongations of the PT ( $*p<0.001$ ) compared with  $PTr = 1$ . (B) Increasing 24-h administration of transfusion products with increasing prolongations of the PT ( $*p<0.001$ ) compared with  $PTr = 1$ . ( $+p<0.001$ ) compared with  $PTr = 1$ . (64)

## 1.11 ROTEM

In the past decade, viscoelastic methods that assess the speed of clotting and the quality of the clot, such as ROTEM<sup>®</sup>—Tem International GmbH, Munich, Germany (Figure 1.11)—have been successfully used to guide haemostatic therapy. The use of viscoelastic screens



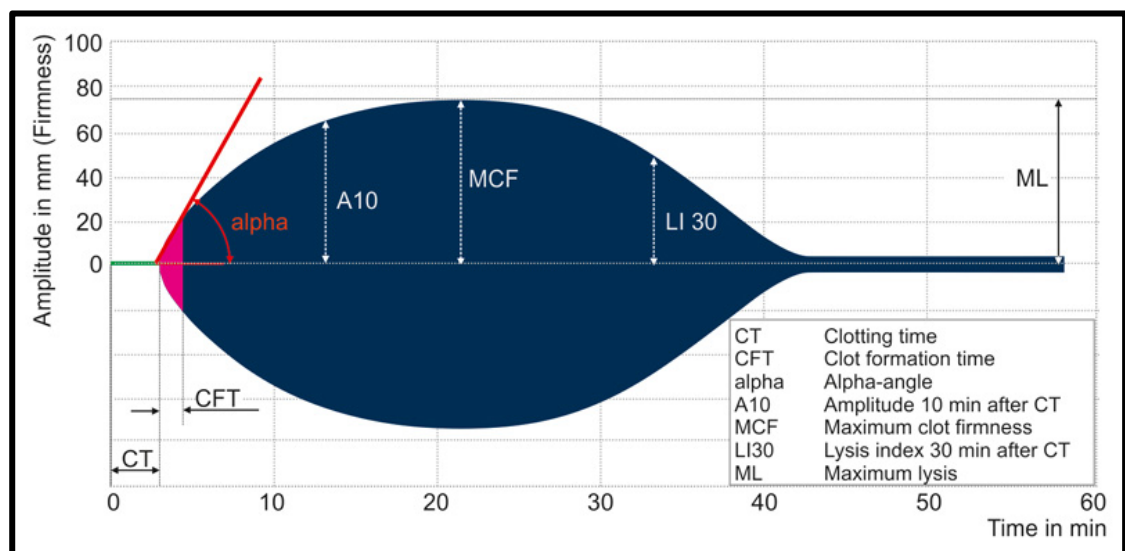
have been evaluated in cardiothoracic surgery (85), obstetric practice (86), inherited bleeding disorders (87), monitoring of haemostatic agents such as activated recombinant factor VIIa (rFVIIa; NovoSeven, Novo Nordisk) (88) and bleeding trauma patients (88-91).



**Figure 1.11. ROTEM (delta).**

ROTEM assesses the viscoelastic properties of blood, also known as “modulus of elasticity” or “clot firmness”, and provides graphical representation of clot formation and breakdown (fibrinolysis). This measurement occurs during the transition of blood from a fluid to semi-solid state and exhibits good correlation with thrombin generation assays (62, 92). Blood is incubated at 37°C in a series of cups with activators of coagulation (such as tissue factor, ellagic acid, and kaolin). The relative contribution of fibrinogen and platelets to clot strength can be evaluated through the inhibition of thrombocytes in ROTEM (FIBTEM).

Measurement of coagulation in ROTEM is performed after the vertical immersion of a plastic pin into the whole blood sample. The pin rotates slowly backwards and forwards at an angle of  $4.75^\circ$ . The oscillating pin is attached to an optical detector. After the generation of the first fibrin filaments between the pin and the wall of the test cup, the rotational range of the pin is reduced. The increasing restriction of the pin's movement is transferred to a graphical display, a plot that shows changes in the viscoelastic properties of the clot over time (*Figure 1.12*). The device has four channels, allowing four tests to be performed simultaneously. Two basic ROTEM tests that use intrinsic activation (INTEM) and extrinsic activation (EXTEM) provide information on the general coagulation status.



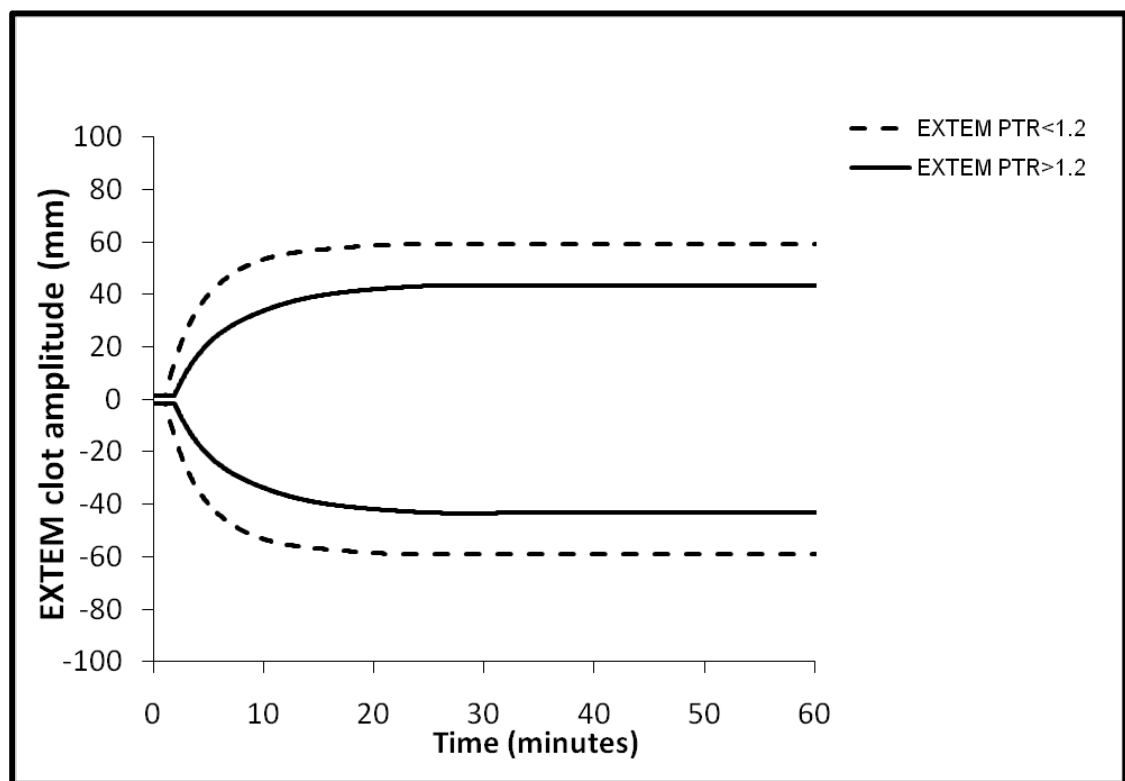
**Figure 1.12. ROTEM Trace.** (*Adapted from ROTEM<sup>®</sup>, Tem International GmbH, Munich, Germany.*)

<b>ROTEM</b>	<b>Description of Parameter</b>	<b>Interpretation of Parameter</b>	<b>Main Factors</b>
<b>CT (seconds)</b> Clotting Time	The CT is the time from the beginning of the test, once the clotting activator is added, until the time when an amplitude of two mm is achieved.	<b>Initiation phase</b>  The CT describes how fast the formation of fibrin begins. This parameter is analogical to the clotting time in a classical clotting test in a laboratory. It is the lag-phase of thrombin generation (dependent on the activity of coagulation factors) clot (amplitude of two mm)	<i>Clotting factors</i>  <i>Anticoagulants</i>
<b>CFT (seconds)</b> Clot Formation Rate	The CFT is the time between a two mm amplitude and a 20 mm amplitude of the clotting signal.	<b>Amplification phase</b>  The CFT describes the next phase of the clotting: the kinetics of the formation of a stable clot through both activated thrombocytes and fibrin.	<i>Clot firmness as influenced by thrombocytes.</i>  <i>Fibrinogen level and its ability to polymerise.</i>
<b><math>\alpha</math>-angle (degrees)</b>	The angle between the middle axis and the tangent to the clotting curve through the two mm amplitude point. It describes the kinetics of clotting.	<b>Thrombin burst</b>  Maximum velocity of clot formation (dependent on platelet function and fibrin polymerisation).	<i>The diagnostic information of this parameter is similar to CFT.</i>
<b>CAx (mm)</b> Clot Amplitude	Mechanical clot quality at fixed time point, e.g. 10 minutes (CA10).	<b>Propagation phase</b>  Clot strength at a fixed time point.	<i>Thrombocytes</i>  <i>Fibrinogen concentration and ability to polymerise.</i>  <i>Factor XIII.</i>
<b>MCF (mm)</b> Maximum Clot Firmness	MCF is the measure for the firmness of the clot and therefore the clot quality. It is the maximum amplitude that is reached before the clot is dissolved by fibrinolysis and the clot firmness falls again.	<b>Maximal clot strength</b>  Determined by platelets, fibrinogen and factor XIII.	<i>Thrombocytes</i>  <i>Fibrinogen concentration and ability to polymerise.</i>  <i>F XIII.</i>  <i>The status of fibrinolysis.</i>
<b>LIX (%)</b>	LIX is ratio of the amplitude to MCF at a given time point after CT.	<b>Clot termination</b>  Percentage clot lysis at a fixed time e.g. 30 minutes (LI30).	

**Table 1.2. Standard nomenclature ROTEM parameters.** (Modified from Tem International GmbH, Martin-Kollar-Strasse 13–15, 81829 München, Germany, 2014.)

Compared to laboratory-based clotting assays, ROTEM is a rapid measure of coagulation. Laboratory clotting screens are of limited value in the management of trauma haemorrhage due to delayed availability of results, poor predictive power for MTs, and inability to quantify clot propagation vs clot lysis or overall clot strength (62).

Recent work in ROTEM within our unit has shown that a threshold of clot amplitude at five minutes of  $<35$  mm can diagnose ATC and predict the need for MT with reasonable accuracy—i.e. with a detection rate of 71% versus 43% for  $PTr > 1.2$  (62). We have also shown that trauma patients with ATC (defined as  $PTr > 1.2$ ) have a “signature” thromboelastogram (*Figure 1.13*).



**Figure 1.13. A computer-simulated signature ROTEM ATC Trace and Non-ATC Trace (62).**

## **1.12 Advantages of ROTEM**

ROTEM can be operated by non-laboratory staff and is able to deliver a dynamic display of clot initiation, quality and lysis. The graphs generated show the degree of clot firmness as amplitude of the signal. The dynamics of clot formation and lysis are displayed as a change in this amplitude over time, providing a graphical representation of all stages of haemostasis, from clot initiation, clot formation to fibrinolysis. Unlike PT and aPTT, ROTEM is able to graphically evaluate the rate of clot propagation vs clot lysis or overall clot strength. Therefore, the speed of detection, whole blood assay and evaluation of clot dynamics make ROTEM an attractive option for the rapid identification of ATC.

Educational programmes with training certifications and telephone support from transfusion experts have been shown to assist physicians in the interpretation of ROTEM traces and the clinical implications of abnormal results. Some hospitals have adopted a remote system, utilising trained technicians in the laboratory to perform ROTEM with results transmitted in real time to monitors in the clinical environment for correlation.

A further advantage of using ROTEM is that it requires a relatively small blood sample. It only uses 300 micro l per test and a pre-set automated pipetting system that ensures accuracy. The ROTEM itself uses simple procedures, and hence is an easy test to learn. Consequently, intra-operator variability is low.

## **1.13 Limitations of ROTEM**

ROTEM has been developed by clinicians independent of haematologists and haemostasis laboratories. Thus, they have yet to be fully scrutinised to provide any large-scale validation and hence clinicians are unable to make decisions based on the metrics created.. Comparison with previous studies is inherently difficult, given the large variation in

sampling protocols and device configuration used to evaluate ROTEM, such as fresh vs citrated blood, activated vs non-activated samples and so on. Commercially available assays are different with varying sensitivities, specificities, accuracies and reproducibility depending on the type and concentration of activator used. Furthermore, massive activation of the coagulation system with tissue factor may mask subtle changes that may occur.

Factors for consideration regarding any near-patient testing are clinical staff training, frequency of maintenance and the need for robust quality assurance. ROTEM users need to fully engage with external quality assessment (EQA) providers to enable a monitoring of the performance of the devices. High staff turnovers and a relatively low incidence of TIC in some centres may limit clinician competency in the timely analysis of ROTEM graphs for bleeding trauma patients. Only major trauma centres that treat a high number of severely injured patients are likely to develop expertise in the ROTEM-guided management and diagnosis of TIC.

Furthermore, the high costs of running a four-channel ROTEM machine are significant when compared to laboratory tests such as PT and aPTT. There is not only the cost of the machine itself to consider, but also the consumables involved in the sample analysis. A solution to this is to only analyse EXTEM and FIBTEM channels, thus reducing the cost, however this has obvious implications for broader analysis.

Finally, in 2014 NICE evaluated three viscoelastometric point-of-care testing devices (ROTEM, TEG and Sonoclot systems), to aid the NHS to decide whether to implement these tools. The published recommendations in 2014 reported that both ROTEM and TEG were used to help detect, manage and monitor haemostasis during and after *cardiac surgery* (93). However in regards to the emergency control of bleeding, NICE concluded there was “*currently insufficient evidence to recommend the routine adoption of viscoelastometric point-of-care testing in the NHS to help detect, manage and monitor haemostasis in*

*bleeding after trauma*” (93). Furthermore they recommended specific focused research into the clinical benefits and cost effectiveness of using viscoelastic point-of-care testing to help with the emergency control of bleeding after major trauma. In particular NICE recommended that future trials should include longer-term follow-up beyond the initial hospital episode, and further research to understand the characteristics of patients at high risk of haemostatic instability in whom viscoelastic testing may be most effective (93) .

## **1.14 Alternatives to ROTEM**

Another viscoelastic coagulation test that could be a possible alternative to ROTEM is thromboelastography (TEG). TEG works on a similar principle to ROTEM, however in TEG, a stationary pin attached to a wire (that can monitor movements) is immersed into the sample. The clot strength will influence the oscillation of the pin and these dynamic changes are converted to a curve.

A major limitation of TEG in an emergency setting has been the sensitivity of the torsion pin to vibration and shock. The oscillating pin in ROTEM is believed to offer greater stability to the device, such that the military have been able to use the system with good effect. The Multiplate analyser is another possible alternative. The Multiplate is a functional analyser of coagulation based on whole blood impedance aggregometry. An advantage of Multiplate is that it shows a high sensitivity towards the effects of platelet inhibitors, such as ASA, clopidogrel and GPIIb/IIIa receptor inhibitors, unlike ROTEM. Since the Multiplate analyser is a fairly new device, only limited data exist regarding its diagnostic power.

Fast, reliable diagnosis, as well as characterisation of TIC, is important and there is increasing evidence that these coagulation monitoring devices are helpful in guiding coagulation therapy for heavily bleeding trauma patients according to their actual needs

(94). In severely injured, bleeding trauma patients, it is crucial to receive rapid information on the patient's current coagulation status. Low MCF in EXTEM, INTEM and FIBTEM has been identified as important determinants of RBC transfusions (95) (62, 75) (96) (97). In one study, Schochl et al. showed that MCF—but not standard coagulation tests (INR, PT, aPTT)—was predictive for blood product transfusions. Leemann et al. showed that low INTEM MCF and low haemoglobin levels were independent risk factors for a MT (>10 U PRBCs). Doran et al., in 2010, demonstrated (in a small cohort of severely injured combat casualties) that admission samples analysed by ROTEM are more sensitive than aPTT and PT in detecting coagulopathy. In patients who received a MT, the PT or aPTT was abnormal in 21% of cases compared to 64% for ROTEM (98).

### **1.15 Historic haemorrhage control strategies in trauma**

Once a patient has arrived in the trauma room, the initial management of multiple trauma patients should always adhere to ATLS® principles and undergo the primary survey of airway (A), breathing (B), circulation (C), neurologic status (D: disability) and core temperature (E: environment) (99). However, patients suffering from severe trauma often arrive in the ED in hypovolemic shock and need large volumes of packed red blood cells (PRBCs) and blood component transfusion to minimise further loss, restore tissue perfusion and achieve haemodynamic stability (100).

Historically, resuscitation strategies in trauma have involved large volumes of crystalloid followed by PRBC infusion. Other blood components such as fresh frozen plasma (FFP), cryoprecipitate (CRYO) and platelets (PLT) were supplemented based on routine laboratory values and at the discretion of ED physicians. The goal of this mode of treatment strategy was to address coagulopathy after the trauma patient has been initially stabilised and the acute resuscitation has been completed. This approach to resuscitation was, until very recently, endorsed by the American College of Surgeons Committee on Trauma and



the ATLS course. The guidelines suggest resuscitating all trauma patients with signs of shock with two or more litres of crystalloid in order to restore normal blood pressure. The pitfall of this methodology is its reactionary treatment of coagulopathy in that it tends to be too late in the clinical course, as dilution, consumption and fibrinolysis is now already deeply rooted (101).

## **1.16 Fluid therapy**

Haemorrhagic shock can be defined as “a condition produced by rapid and significant loss of intravascular volume, which may lead sequentially to hemodynamic instability, decreases in oxygen delivery, decreased tissue perfusion, cellular hypoxia, organ damage, and death” (102). Haemorrhagic shock can be rapidly fatal. Patients presenting with hypotension (systolic blood pressure less than 90 mmHg), tachycardia (heart rate greater than 100 beats per minute), and obvious blood loss are readily identified as being in a state of haemorrhagic shock. In the case of haemorrhagic shock, fluid infusion represents the main treatment to improve perfusion, but aggressive replacement increases dilution coagulopathy and interstitial oedema and impairs microcirculation, thus worsening oxygenation (103). Furthermore, pre-hospital administration of high volumes (>3 L) of crystalloid or colloid fluids has been shown to be independently associated with a worse coagulation profile at ED admission (104). In 1985, a retrospective review by Hewson et al. of 68 massively transfused patients found that coagulopathy was common after crystalloid administration and that PTT correlated with the volume of crystalloids given (105). Thus, vigorous fluid resuscitation not only increases blood pressure, the effect of which increases hydrostatic forces on newly formed clots, but also dilutes clotting factors and haemoglobin and reduces body temperature. All of these effects could promote further bleeding and impair end organ perfusion (106).

## **1.17 Permissive hypotension**

Approximately one third of trauma deaths occur because the victims bleed to death within the first hours of their injury (107). For almost half a century, high-volume fluid resuscitation strategies have typically been used by trauma surgeons and emergency medical personnel in an attempt to reverse haemorrhagic shock by replacing lost blood with intravenous (IV) fluids or transfusions. Hypotensive resuscitation, also known as permissive hypotension (PH), has been proposed as an alternative to the current standard of care. Although PH is only now evolving into an integral part of DCR, the practice itself is not a new concept. Walter Cannon and John Fraser remarked on it as early as 1918, when serving with the Harvard Medical Unit in France during World War I (108). The main feature of PH, in contrast to standard fluid resuscitation, is that it uses less IV fluids and blood products during the early stages of treatment for haemorrhagic shock. In essence, PH resuscitation describes a process that minimises administration of fluid resuscitation until haemorrhage control has been achieved.

Scientific attempts to examine outcomes for PH after serious traumatic injury have been mixed. A well-known randomised control study of trauma patients with truncal injuries, published by Bickell et al. in 1994, concluded that there was a benefit to delaying aggressive fluid resuscitation until after operative intervention and surgical haemorrhagic control had been established (109). This group compared mortality rates of patients who received immediate vs delayed administration of IV fluids and discovered improved survival, fewer complications, and shorter hospital stays in the delayed group. They demonstrated that, regardless of the victim's blood pressure, survival was better in their urban "scoop and run" rapid transport system when no attempt at pre-hospital resuscitation was made (109). A study produced in 2006 by Hirshberg et al. utilised computer modelling to demonstrate the effect that the timing of resuscitation has on bleeding. It showed that an early bolus delayed haemostasis and increasing blood loss, while a late bolus triggered

rebleeding (110). Moreover, the limited use of fluids during resuscitation efforts is in direct opposition to guidelines recommended by the American College of Surgeons Committee on Trauma and the ATLS protocol (108).

There have been theoretical concerns regarding the safety of hypotensive resuscitation. These are largely based on the possible harmful effects of decreased oxygen delivery to the various tissues of the body, a by-product of haemorrhagic shock. On the one hand, maintaining a MABP that is too low could result in inadequate perfusion, acidosis and subsequent organ failure, with potentially catastrophic outcomes. On the other hand, critics of aggressive fluid resuscitation argue that maintaining a raised or “normal” blood pressure in the face of exsanguination can result in equally catastrophic outcomes secondary to exsanguination and perpetuate the lethal triad of hypothermia, acidosis, and coagulopathy (111) in addition to breakdown of the newly developed fibrin clot. Morrison et al. (2011) prospectively examined the 30-day morbidity and mortality rates for the first 90 patients enrolled in a randomised controlled trial of hypotensive resuscitation, with the primary aim of assessing the effects of a limited transfusion and IV fluid strategy (107). These researchers showed that the PH group had significantly lower mortality in the early postoperative period and a non-significant trend for lower mortality at 30 days. When examining coagulopathy, they report that, among the PH group, there was a significantly lower international normalised ratio as compared to those in the higher mean arterial blood pressure (MABP) group, indicating a less severe coagulopathy. These preliminary results provide convincing evidence that supports the continued investigation and use of hypotensive resuscitation in a trauma setting.

## **1.18 Damage control resuscitation**

The most important aim when treating an exsanguinating patient is the recognition and immediate control of bleeding. It is also possible to rapidly identify patients who are highly

likely to develop ATC at admission and promptly, aggressively and simultaneously treat hypothermia and acidosis. The technique to achieve this, developed by clinicians, is known as Damage Control Resuscitation (DCR) (39) and is a treatment strategy that targets the conditions that exacerbate haemorrhage in trauma patients.

The term was originally coined by the US Navy in reference to techniques that maintained a battleship's functional integrity after it had sustained serious or critical damage, and refers to the guidelines developed for combat casualties suffering substantial bleeding in Iraq and Afghanistan (112). Since then, "damage control" has been adapted to truncating initial surgical procedures on severely injured trauma patients, thus providing only those interventions necessary to establish haemostatic control and to focus on re-establishing a survivable physiologic status.

DCR, a concept that has been popularised by the military, is now being studied in a civilian setting. In recent times, traditional methods of resuscitation of severely injured patients with exsanguinating haemorrhage have come under increasing scrutiny for their inadequacy in correcting the acidosis, hypothermia, and coagulopathy seen in this population of patients. Direct treatment of coagulopathy has been relatively neglected, unfortunately viewed as a by-product of resuscitation, haemodilution, and hypothermia, and delayed by blood banking logistics (113-116). Conventional resuscitation practices for damage control mainly focus on rapid reversal of worsening acidosis and prevention of hypothermia, and surgical techniques focus on controlling haemorrhage and contamination from perforated viscera. The concept of DCR has its foundations in the assumption that a coagulopathy is present very early in the clinical course after injury, and hence, earlier interventions to correct it in severely injured patients may lead to improved outcomes (108). The temporarily stabilised trauma patient then undergo continued resuscitation and aggressive correction of their physiology in the intensive care unit (ICU) before returning to the operating room (OR) for the definitive repair of their injuries. DCR is therefore a structured

intervention that begins immediately after initial assessment in a pre-hospital setting and ED and progresses through the OR into the ICU. It comprises two main components, the first being that resuscitation is limited to keep blood pressure at approximately 90 mmHg, preventing renewed bleeding from recently clotted vessels due to increased hydrostatic force. Second, intravascular volume restoration is accomplished by using thawed plasma as a primary resuscitation fluid in a high ratio to PRBCs, usually provided in packs known as MTPs (39).

### **1.19 Massive transfusion**

It is estimated that 10% of military trauma patients and 3% to 5% of civilian trauma patients receive a massive transfusion (MT), which are generally defined as more than 10 U of PRBCs within 24 h of the start of treatment (101). Although this represents a small cohort of patients in real terms, it accounts for 75% of blood utilisation in busy Level 1 trauma centres (62). Furthermore, this direct focus on the sole replenishment of PRBCs over 24 h does not address a significant subset of patients who would likely benefit from additional blood component therapy (i.e. over a shorter interval). In separate studies, both Moore et al. and Holcomb et al. have demonstrated that patients receiving 10 U of PRBCs in the first six hours after injury had a higher rate of mortality than those receiving the same quantity of PRBCs over a 24-hour period (117).

Historically, massive transfusion protocols (MTPs) have been reactive to the problem of traumatic coagulopathy. Until recently, most published guidelines advocated the sequential replacement of PRBCs with crystalloids prior to clotting products and platelets and only after a laboratory confirmed derangement, i.e.  $PT > 1.5$  (118). However, as mentioned previously, laboratory-guided component therapy has a limited use in guiding treatment in exsanguinating patients. Furthermore, there can be a significant interval between when the blood is drawn to when the result is available to the trauma team. In addition, once the

decision has been made by the treating clinical team to transfuse plasma or platelets, more time (30–60 min) is lost while waiting for these products to thaw. In the severely injured, exsanguinating patient, this is a missed opportunity to treat or prevent TIC.

Many institutions have embraced the concept of “proactive” resuscitation and the early use of PRBCs and blood components. This evolution stems from DCR: to expeditiously address the specific issues of rapid exsanguination and ATC, a targeted delivery of blood components in predefined ratios has been proposed and has given rise to the nomenclature of major haemorrhage protocols (MHPs) (39, 113). These MHPs are a formulaic protocol which are activated on the basis of clinical need and hence, not dependent on laboratory triggers such as PT results.

## **1.20 Transfusion strategies for trauma haemorrhage**

Almost 80% of all trauma deaths that occur in the operating theatre are a result of haemorrhagic shock and exsanguination (29). Large volume blood and blood product replacement is required to maintain oxygen-carrying capacity and augment clot formation (82). Rates of MT in civilian trauma practice are less than 5%, but transfusion medicine has undergone a paradigm shift in recent years, following numerous reports of improved outcomes with early high-dose plasma therapy (119). Traditionally, MTPs have been reactive to the problem of traumatic coagulopathy. Until recently, most published guidelines advocated the sequential replacement of PRBCs with crystalloids prior to clotting products and platelets, and only after a laboratory confirmed derangement, e.g.  $PT > 1.5 \times \text{normal}$ ,  $\text{platelets} < 50 \times 10^9/\text{L}$  (81, 82, 120). Computer modelling of major haemorrhage and MTPs offered reason to question the rationale and efficacy of restrictive transfusion practice. In 2005, Ho et al. demonstrated excessive coagulation factor dilution with delayed low-dose FFP that required at least 1:1 therapy to reverse the coagulopathy (121). Using an alternative simulation, Hirshberg et al. (2003) reported optimal replacement

ratios of 2:3 and proposed the concurrent use of plasma with an initial PRBC transfusion to prevent prolongation of clotting times (122). A large number of retrospective clinical studies have supported the results of these theoretical models, and several survival benefits have been seen following the adoption of aggressive transfusion strategies (119). Borgman et al. were the first to draw an association between the ratio of FFP:PRBCs and mortality (123). Casualties who received ratios of  $<1:4$  had a 65% mortality rate, compared to a mortality rate of 19% in those who received  $>2:3$  FFP:PRBCs. Similar analyses have been conducted using both military and civilian trauma registries, the majority of which have advocated ratios approaching 1:1 (82). Survivor bias is a major confounding factor in these retrospective studies as patients have to live long enough to receive a high ratio. Interestingly, recent work within our group has shown that the haemostatic effects of FFP:PRBCs may be maximal at red cell ratios of 1:2, again with 1:1 not conferring any additional benefit (124).

## **1.21 Full whole blood**

As part of DCR, severely injured trauma patients were historically transfused with full whole blood (FWB), the resuscitative fluid of choice. Full whole blood was historically used in transfusion until it fell out of favour in the middle of the twentieth century due to its side effects and the convenience of component therapy for treating other non-traumatic diseases (108). Resuscitation strategies in the 1980s replaced the use of whole blood therapy with component therapy. In theory, whole blood replaces all the blood components lost to trauma haemorrhage, including platelets and fully functional coagulation proteases. Furthermore, the components of FWB are more functional than their stored counterparts. Separating blood into components results in dilution and the loss of about half of the viable platelets (108). Moreover, logistically, FWB provides the advantage of being readily available and requires no delay to account for thawing. However, it does require the presence of a ready and willing donor pool. It should be noted that transfusion with FWB,

as well as with component therapy, does carry its associated risks. Mortality in transfused trauma patients, even when controlled for other risk factors, has been well documented (39). Specifically, this relates to transfusion related acute lung injury (TRALI), which occurs in one in every 100,000 transfusions and is now the leading cause of mortality after blood product administration. TRALI is a life-threatening antibody-mediated event, most often seen during the administration of FFP. Other complications include multi organ failure (MOF) due to immunogenic transfused cells or infection. Infections are a common and significant sequelae of major traumatic injury and transfusion. In 2002, Claridge et al. prospectively evaluated the relationship between infections in 1,953 trauma patients and the transfusion of PRBCs within the first 48 hours of admission. They demonstrated that infection rates were four times more likely in transfused patients than in those who did not receive transfusion. Although transfusions are frequently required in the trauma setting, they should be administered appropriately and with no more PRBCs than absolutely necessary (125).

Formula-driven MHPs are becoming commonplace in trauma haemorrhage management (126), although little is known regarding the effect MHPs have when compared directly with MTPS. A knowledge gap exists on the effects MHPs' blood components have on TIC and *in vivo* coagulation factor concentration.

## **1.22 Fresh frozen plasma**

Damage control resuscitation aims to directly address TIC through the early and aggressive administration of blood products to severely injured trauma victims. It is proposed that early and sustained administration of FFP can help correct the state of depleted coagulation factors common in a bleeding patient. However, the evidence for its efficacy in managing massive haemorrhage is limited (127). There are also inherent logistical obstacles to providing FFP to a trauma patient in a timely fashion. As the name suggests, a FFP is



plasma that is frozen at -18°C within eight h of being drawn from the donor pool. Using this method of freezing and storing plasma has the advantage of preserving all coagulation factors at their *in vivo* activity levels. These factors therefore remain stable during storage. Unfortunately, the disadvantage of this method is that the emergency transfusion is complicated by a 10–20 min delay while the units are thawed. Thus, it cannot always be available to a massively haemorrhaging patient during the crucial first minutes of DCR.

Some centres have moved towards using alternative plasma products, such as thawed plasma and liquid plasma. These units are stored in liquid form and can be provided to an exsanguinating trauma patient immediately, without the need for thawing. Some loss of clotting factors has been reported when plasma is stored in liquid form, particularly loss of the “labile” factors V and VIII. Alternatively, thawed plasma can be considered equivalent to FFP. It is stored in liquid form for a maximum of five days after it is thawed. At the end of five days, coagulation factors other than factors V and VIII maintain 70% to 80% of their original activity levels, and fibrinogen levels are unchanged. The levels of factors V and VIII are reduced to 65% activity, but still within the haemostatic range (108). Thawed plasma has an average five-day shelf life; therefore it can be difficult to keep an adequate supply of thawed plasma on hand in the ED, in particular for the scarce “universal donor” type, AB. One possible option is liquid plasma, which has a shelf life of between 26 to 40 days, depending on the preservative used.

FFP is thought to contain all the coagulation proteases, fibrinogen (0.9g/unit) (128), and physiological anticoagulants (e.g. protein C, protein S and plasma proteins (129)), but the precise quantities of each vary from unit to unit, as there is no standardisation of individual FFP units. Within the trauma setting, larger quantities of FFP are recommended (greater than 10–20mg/kg) for a beneficial effect on the exsanguinating patient, but there are no published evidence-based guidelines as yet.

There is much controversy over the ideal FFP:PRBC ratio in the trauma setting. In 2002, while describing the effect of fluids on coagulation, Hirshberg et al. concluded that to avoid coagulopathy, RBCs and FFP must be given in a 3:2 ratio (122). This concept further evolved to the use of a higher 1:1 FFP:PRBC ratio, although this is based largely on the evidence acquired from the military data regarding the management of combat casualties. Borgman et al. compared mortality rates associated with varying ratios of FFP to PRBCs in the management of trauma seen in the Iraq conflict (123). They performed a retrospective review of 246 patients at a US Army combat support hospital, each of whom received a MT. Three groups of patients were created according to the plasma to RBC ratio transfused during MT, and the mortality rates and the cause of death were then compared among groups. The researchers concluded that, in patients with combat-related trauma requiring MT, a high 1:1.4 plasma to PRBC ratio is independently associated with improved survival and hospital discharge, primarily by decreasing death from haemorrhage (123).

There have also been numerous civilian observational studies that report good outcomes across a range of different blood product ratios (40, 108, 117). The largest observational transfusion study was of 905 bleeding trauma patients and was conducted in 10 Level I trauma centres in the US: it is known as the prospective observational multicentre major trauma transfusion (PROMMTT) study. In this PROMMTT study, investigators conducted a prospective group study documenting when FFP and PRBC transfusions were administered during active resuscitation and patient outcomes. Clinicians generally delivered transfusion ratios that cumulated in the range of 1:1 or 1:2, which shows a clinical equipoise for these two ratios (130).

Transfusion of higher ratios of FFP:PRBCs is not without risk (51, 131). In 2008, Sperry et al. conducted a multicentre prospective cohort study evaluating clinical outcomes in 415 blunt injured adults with haemorrhagic shock. They concluded that there was a higher risk of acute respiratory distress syndrome in those patients who required  $\geq$ FFP:PRBCs of

1:1.5. Furthermore, aside from the transmission of viral or bacterial pathogens, which should be effectively screened out prior to transfusion, blood products are independently associated with numerous adverse effects (131). Various groups have reported that immunomodulation and the risks of noscomial infection are more prevalent in transfused trauma patients (132, 133), and FFP itself is an independent risk factor for acute lung injury (ALI) and MOF (51). However, certain groups have questioned whether a more liberal transfusion policy actually controls bleeding earlier and therefore reduces exposure to blood and blood products (134). Blood components are a limited resource. Increased demands both on the availability and quantity of FFP are likely to impact heavily on transfusion services. Although there is a rapid uptake of newer transfusion strategies that promote a higher ratio component delivery, there is a lack of high-level evidence on the effect of these strategies on the coagulation profile *in vivo*.

## **1.23 Platelets**

There is a widely accepted consensus that, in severe haemorrhage, the platelet count should be maintained above  $50\text{--}100 \times 10^9/\text{L}$  in polytrauma or central nervous system injury (68, 76, 135). A dilutional thrombocytopenia (platelet counts  $<50\text{--}100 \times 10^9/\text{L}$ ), which is thought to be the major cause of microvascular bleeding, tends to develop only after MT patients received 18–20 units of stored whole blood (40). Further to the thrombocytopenia, intrinsic platelet function is impaired by both acidosis and hypothermia, which can develop in severely injured patients who require MT.

Historically, it has long been thought that a declining platelet count usually requires intervention much later than a plasma deficit (136). Although the platelet count can be easily determined, there are no validated practical methods to rapidly assess the function of native platelets. Several military retrospective studies have reported improved outcomes after earlier transfusion of increased amounts of blood products (123, 137). In 2008,

Johansson et al. reported that increased amounts of plasma and platelets transfused in the OR in fixed ratios for patients with ruptured aortic aneurysms improved chances of survival (44% vs 66%,  $p < 0.05$ ). Furthermore, a platelet count of  $\geq 100 \times 10^9$  upon arrival in the ICU was associated with improved survival. However, it remains to be seen if similar results can be achieved in a civilian trauma population, with different injury patterns, increased comorbidities, and without fresh whole blood transfusions.

In 2008, Holcomb et al. hypothesised that higher PLT:PRBC and FFP:PRBC ratios would result in decreased early haemorrhagic death and this benefit would be sustained throughout the ensuing hospital stay (40). Over a one year period, this civilian study examined records of 467 MT trauma patients transported from the scene to 16 level 1 trauma centres. Using multivariate logistic models, their results showed that plasma and platelet to PRBC ratios and ISS were predictors of death at six hours, 24 hours, and 30 days. Specifically, patients with high PLT:PRBC ratios ( $\geq 1:2$ ) had an increased 30-day survival compared to those with low PLT:PRBC ratios (59.9% vs 40.1%,  $p < 0.01$ ) (40). More recently, the same group published results from Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial, a pragmatic randomised controlled trial of 680 severely injured patients, where half were randomised into 1:1:1 blood product ratios and compared to those receiving 1:1:2 blood product ratios (138). In contrast with earlier reports, for patients with major haemorrhage, early administration of plasma, platelets, and RBCs with either ratio did not result in significant differences in 24-h mortality rate (1:1:1: 12.7% vs 1:1:2: 17.0%  $p = 0.12$ ) or 30-day mortality rates (1:1:1: 22.4% vs 1:1:2: 26.1%  $p = 0.26$ ). However, more patients in the 1:1:1 cohort achieved haemostasis and fewer died due to early exsanguination.

## 1.24 Fibrinogen

Fibrinogen is the primary substrate of the coagulation system and is fundamental to haemostasis. Profound acidosis, severe hypothermia ( $<32^{\circ}\text{C}$ ) and haemodilution have been shown to lower fibrinogen (139, 140). In 2006, Martini et al. reported that acidosis compromised the clotting process, while accelerated fibrinogen consumption reduced fibrinogen levels in experiments conducted on swine with ATC (141). There have also been small, retrospective clinical studies that suggested a deterioration in fibrinogen levels (88, 142); however, these levels have not reached values that would trigger fibrinogen replacement. To maintain the integrity of the coagulation function, it is recommended that fibrinogen is replaced when it falls below 0.8–1.0g/L (81).

Although fibrinogen replacement has been used as first-line treatment for trauma-induced coagulopathy, and recent MT guidelines suggest maintaining higher levels of fibrinogen (above 1.5g/L), supporting evidence is weak (82, 143, 144) and not without limitations. Early clinical studies have pointed towards fibrinogen supplementation as a means to improve outcomes for trauma haemorrhage by improving clot strength (145), reducing blood loss (146) and increasing survival (88, 147). Likewise, two observational cohort studies (148, 149) have also reported a reduction in mortality for those patients who receive higher fibrinogen content during trauma haemorrhage therapy. To add weight to these findings, it is known that hypofibrinogenaemia is a key component of ATC (75) and occurs early during major blood loss (150). Furthermore, the CRASH-2 randomised controlled trial has not only confirmed the importance of tranexamic acid (TXA) and antifibrinolytics as a means to improve clinical outcomes (151), but, more importantly, has also shown that it is vital to deliver haemostatic therapy early (i.e. within three h of injury) (152).

Fibrinogen supplementation with either Cryoprecipitate (CRYO in the UK or US) or fibrinogen concentrate (in Europe) is often delayed or considered second-line treatment in

the empiric delivery of haemostatic coagulation therapy (153). The estimated expected fibrinogen doses transfused in each blood component are PLT – 0.4g; FFP/ – 0.9g; and CRYO – 2g for each pool/unit transfused (128). Our group has shown that a low-admission fibrinogen level was independently associated with ISS ( $p<0.01$ ), shock ( $p<0.001$ ) and pre-hospital fluid volume ( $p<0.001$ ) (154). This prospective study showed that fibrinogen supplementation during transfusion maintained, but did not augment, fibrinogen levels. Furthermore, our group reported that administration of CRYO was associated with improved survival. In addition to this, ROTEM parameters correlated with fibrinogen levels and *ex vivo* fibrinogen administration reversed coagulopathic ROTEM parameters. Our group also showed that the fibrinogen level was an independent predictor of mortality at 24 h and 28 days ( $p<0.001$ ) (154). Further work is required to ascertain the true effect of fibrinogen replacement on the coagulation profile during DCR.

## **1.25 Transfusion practice in trauma**

In recent years, there has been a shift in trauma haemorrhage management practices. Older Massive Transfusion Protocols (MTPs) that *treated* the coagulopathy associated with MT have been replaced by new Major Haemorrhage Protocols (MHPs) that *predict* those patients who will go on to receive a MT and thus protect haemostatic potential.

Unsurprisingly, the demand for blood products when managing bleeding trauma patients depends a great deal on adequate and timely blood transfusion support (155). Transfusion requirements in the acute resuscitative trauma setting can be extremely challenging and demanding on the resources of a blood bank. Blood and its components are a scarce and costly resource and demand is anticipated to increase with an ageing populations (156, 157) (158). Transfusion laboratories try to maximise delivering the correct blood component from the blood bank to the right patients in a timely manner. The easiest way to assure the

timely availability of blood is to have an appropriate inventory on the shelf at all times; however, this is impossible due to the shortage of blood products across the NHS.

In the trauma setting, there is a need for large volumes of blood components for those with known or suspected haemorrhage. Indeed, the most severely injured and actively bleeding patients can go on to require a massive blood transfusion (i.e. receiving more than 10 units of PRBCs) (159). Therefore the blood transfusion laboratory must provide large amounts of blood products promptly, but avoid excessive waste or over provision, which would expose the recipient to the unnecessary risks of immediate, early and late transfusion complications. This threshold identifies a group of patients with severe injury and large resource requirements, and much recent research has been directed at this population. It is this subset of trauma patients that creates the greatest burden on blood transfusion resources (159).

In 2004, Como et al. conducted a retrospective study of over five thousand trauma patients at a US Level 1 Trauma centre in order to describe categorical associations between demographic data, ISS, transfused products, and patient outcomes. In this study, special attention was paid to the groups receiving greater than 10 U of PRBCs. These researchers concluded that although 5,219 PRBCs were transfused and only 3% of patients received more than 10 U, this group of 3% went on to receive 71% of all PRBCs issued by the transfusion laboratory (159). Furthermore, this subgroup of patients also had a high mortality rate of 39% (159). Therefore, the blood transfusion laboratory must promptly provide large amounts of appropriate blood products and communicate effectively with the trauma team. This allows for the early recognition of patients with MT needs and ensures their blood product requirements are met without excessive waste or over provision.

Over the past decade, there has been extensive research into the optimum blood transfusion strategy for exsanguinating trauma patients (68, 124, 130, 146, 160, 161). However, little

information has been gathered on the logistical issues posed by these trauma patients on transfusion services (155). Specific challenges present themselves to the all blood banks that support trauma patients. These include how to correctly identify those patients that are in need of a MT, how to minimise temporal delays associated with blood test results, how to facilitate effective communication between the blood bank and the clinical team, how to rapidly deliver blood products to patient care locations, and finally, how to remain organised and well-coordinated in the acute trauma setting (155). A possible solution to these issues was the introduction of MTPs.

MT protocols originally evolved from elective (planned) surgery and then translated to the trauma setting in conjunction with transfusion services. They included the activation of additional personnel, employment of rapid infusion systems, automatic thawing of plasma, and changes in the crossmatching policy (159). Numerous studies have supported the logical notion that MTPs designed to give RBCs and coagulation factors (i.e. plasma and platelets) in pre-specified ratios are associated with increased survival, faster time to transfusion (118), reduced incidence of sepsis and MOF, decreased length of stay (162) and reduced blood product usage (163).

In the first instance, it is important how the MTP is initiated. One common approach is that only a key point person can initiate the protocol; this is frequently the lead trauma surgeon. The inherent disadvantage of this single MTP-triggering personnel is that there may be a delay in activating the protocol until the trauma surgeon arrives on the scene. More recently, there has been a shift towards a range of individuals who can perform protocol activation. This can vary among different select groups, such as emergency room physicians or pre-hospital teams whose primary aim is to recognise that the patient is at risk of massive haemorrhage.



While the terms have been used interchangeably, there are clear differences between a MTP and the more recent MHPs. An MHP utilises the principles of DCR to aggressively prevent TIC with high ratios of blood products early on in the clinical course of the severely injured trauma patient. In contrast, MTPs *treat* TIC. Goal-directed MHPs aim to simultaneously improve blood product administration while decreasing any waste of the precious resource.

The biggest challenge for blood transfusion services is supplying PRBCs, together with FFP, PLT and CRYO to the exsanguinating patient within a short time frame. In the trauma setting, it is vital that large volumes of blood products reach the patients early in their clinical course. Transfusion of high volumes of PRBCs prior to other blood products may worsen early coagulopathy, as well as increase mortality and blood product waste. Factors that contribute to this are the distance of the transfusion laboratory to the ED, time taken to process blood results, and the thawing of blood products. Unlike PRBCs and platelets, which can be issued to the patient “off the shelf”, FFP can take anywhere up to 30 min to thaw. This can lead to a situation in which the rapidly exsanguinating patient may have received 10 U of packed PRBCs. A possible solution is the introduction of MHPs that account for an empirically predetermined blood product pack with high ratios of thawed FFP:PRBCs. This is used in the ED as part of the initial deployment of blood products. The newly thawed FFP can be used for up to 24 hours afterwards and, furthermore, it can be designated as “thawed plasma” and stored for four days without any significant loss of coagulation factor activity. Once the protocol is activated and the initial haemorrhage packs are used, plasma should be rapidly thawed as units are issued by the transfusion service to allow them to “keep ahead” of expected use in the rapidly exsanguinating patient (164). With a standard operating procedure in place for using thawed plasma, the wastage of plasma should be minimal, but there is little evidence to support this.

Currently, data directly comparing older MTPs to newer MHP strategies in the trauma setting is limited. Increasingly, FFP:PRBC and PLT:PRBC ratios are being used as

surrogate measures of effectiveness for a protocol. However, there is also a need to monitor the amount of blood products that are wasted during trauma. Trauma is among the largest consumers of blood components (165) and these are scarce resources that need to be managed efficiently. “Wasted units” are a crucial parameter to track within trauma (164), as little is known about the financial impact of unused and wasted blood components..

## **1.26 Summary**

In conclusion, there is a knowledge gap regarding newer transfusion algorithms when using DCR principles to target trauma haemorrhage. Furthermore, little is known about the effect of MHPs on blood delivery, blood component waste and patient outcomes when directly compared to out-dated MTPs. Moreover, there is insufficient research investigating the effectiveness of current DCR strategies in addressing TIC in severely injured, bleeding trauma patients. Finally, there is an inadequate understanding regarding how these strategies affect the coagulation profile *in vivo* or how effective they are as a resuscitation tool altogether.

## **1.27 Hypotheses and aims**

The overall research objective of this thesis is to investigate the effect of a DCR strategy on TIC.

**The individual hypotheses are detailed below:**

- 1. Major haemorrhage protocols improve blood product administration and reduces waste compared to traditional massive transfusion protocols.**

- 2. TIC deteriorates during acute trauma haemorrhage.**
- 3. Blood component therapy improves trauma haemorrhage.**
- 4. Blood component therapy maintains coagulation factor concentrations during trauma haemorrhage.**

Each of the aims will be individually addressed within the experimental Chapters 3-6.

## CHAPTER TWO

# **Methods**

The body of work included in this thesis includes patients and used blood samples that I collected as part of the on going prospective study at the Trauma Sciences Group. This ACIT II study received its original ethical approval from the East London and City Regional Ethics Committee on 13 November 2007 (07/Q0603/29) and I wrote the subsequent ethics and protocol amendment that was approved in June 2010. Data that I collected was combined with that already available within our group. My research complied with the Declaration of Helsinki. The ACIT II study was designed to identify the clinically significant mechanisms and pathways by which the inflammatory and coagulation pathways are activated immediately after major trauma, how they lead to clinical coagulopathy and transfusion requirements, as well as produce organ injury, and how they affect outcomes in terms of organ failure and death.

## **2.1 Study design**

This was a prospective cohort observational study of 810 trauma patients admitted to the Royal London Hospital (RLH) from June 2008 until June 2013. My study followed the clinical course of severely injured trauma patients identified on admission to the ED until their discharge, death or day 28. Data was combined with those collected by the previous research group. Blood samples drawn were analysed using ROTEM and assayed for markers of activation of the coagulation and inflammatory systems. Coagulation proteins were assayed and subsequently correlated with study participant injuries, their resulting physiological disturbances, and their subsequent clinical course and outcome.

## **2.2 Study participants**

The analysis of the trauma service registry provided information on the rate and volume of major trauma patients treated at the RLH. On average, there are 1,500 trauma team activations per year at the RLH. I extrapolated data from the RLH trauma registry to show

that the expected study population would comprise a convenience sample with an injury severity distribution of approximately 35% severe, 55% moderate and 7% minor injury over the three-year study period. I aimed to recruit severely injured, actively bleeding trauma patients who required on going resuscitation with blood products from June 2010 to June 2013. These patients were known as “Code Red” patients and form the basis of the body of work presented in this thesis. The specific inclusion criteria for Code Red patients are detailed below.

Severely injured trauma patients are a challenge to recruit to studies owing to the unplanned nature of care, unpredictable work patterns, the emergent nature of treatment and complexities in the consent process. This is further complicated by the high early mortality rate. To this end, I was present at the hospital between 08:00 to 22:00, five days a week, in order to recruit this cohort of patients. I was also present at the hospital on alternate weekends for patient recruitment. My contact details were left within the ED department so I could be contacted at any time during the first two years. Based on average trauma calls within this time frame, it was predicted that a convenience sample of approximately 100 severely injured Code Red patients would be recruited during the study period.

### **2.2.1 Inclusion criteria**

The following criteria are used by the ED clinical team to determine the need for trauma team activation. In order to access the study participants, I carried a bleeper/pager/alert device, which enabled my attendance at the trauma team resuscitation while at hospital. If I was not on-site at the hospital, a member of the ED team would contact me by long-range pager or telephone.

- GCS<14 or RR<10 or <29 or Systolic BP<90.
- Chest trauma with altered physiology.

- Person hit by train.
- Amputation proximal to wrist or ankle.
- Occupant ejected from vehicle.
- Fatality in same vehicle as occupant.
- Suspected pelvic fracture.
- Person trapped under vehicle.
- Suspected open or depressed skull fracture.
- Fall from >2 metres.
- Polytrauma with burns.
- Penetrating trauma (neck to groin or proximal to elbow/knee).
- Explosions.
- Industrial accidents.

### **2.2.2 Exclusion criteria**

- Age <16 years.
- Patients transferred from other hospitals.
- Patients presenting more than two h after time of injury.
- Patients who received more than 2,000 ml of intravenous fluids prior to arrival in the ED.
- Patients with burns >5% of their total body surface area.
- Patients taking anticoagulant medication other than aspirin (<650mg/day).
- Patients with a known bleeding diathesis.
- Patients with moderate to severe liver disease (Child's classification B or C3).

### 2.2.3 Code Red

The most severely injured trauma patients that required ongoing resuscitation were deemed Code Red patients by the TTL. This subset of patients had specific inclusion criteria and are the focus of this thesis:

- Systolic blood pressure <90 mmHg.
- Poor response to initial fluid resuscitation.
- Suspected active bleeding.

## 2.3 Consent process

This study focuses on the very early post-injury phase, and thus, the majority of severely injured trauma patients are either unconscious from a traumatic brain injury or haemorrhagic shock, intubated in the pre-hospital phase of their care, or acutely intubated in ED. These patients were the core population of my research study as it is these patients with severe traumatic injury who will go on to receive a massive blood transfusion. Furthermore, patients who are conscious will have just experienced a major psychologically disturbing event, be in unfamiliar surroundings, and in varying degrees of pain. It is for the above reasons that patients may not be able to comprehend, or it may be inappropriate to discuss the details of a complex research study at this point in time. If the aforementioned patients were deemed unable to consent for themselves, a **professional** legally authorised representative (**PrLAR**) was asked to give permission to enrol the patient onto the ACIT II study. This is in line with the Mental Capacity Act of 2005 that outlines the hierarchy of consent for recruitment of trauma patients to a study. For the first blood draw, as part of the trauma team resuscitation, the PrLAR will be the TTL who is independent from the research protocol. These PrLARs were either Emergency Medicine Consultants or Specialist Registrars and have attended a full brief of the ACIT II study.



Their understanding of the study and the consent process has been documented. They attended six monthly reviews to ensure that they were kept informed of the research progress. If a family member or close friend was available at this point, they could assume the role of **personal** LAR (**PeLAR**), but, often in the case of trauma, the patient arrives at hospital with no next of kin.

Obtaining consent from a patient in the initial phases of trauma evaluation has inherent difficulties, and has the ability to seriously compromise patient care. It was for this reason that only when patients were conscious and had *relatively* minor injuries, such that they were able to comprehend the research protocol and its implications, informed consent was obtained as soon as possible in the ED. A trauma patient can only give informed consent to take part in a clinical study if their decision is given freely after they have been informed of the nature, significance, implications and risks of the trial. Details of the consent forms and patient information sheets are detailed in *Appendices 1–6*.

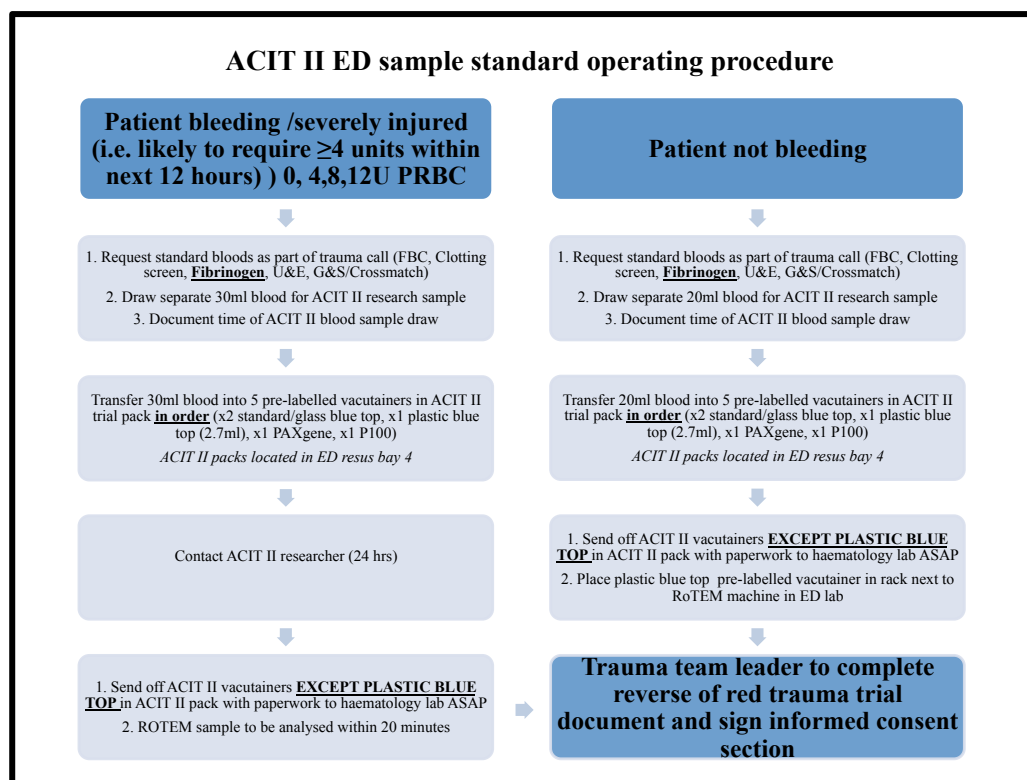
In cases where patients remained unidentified, the police and hospital social workers continued to assist me in identifying the patient. Daily attempts to locate family members to discuss the patient's condition and enrolment onto the ACIT II study were made. All of these attempts were clearly documented in the patient's medical record notes and in my own research consent log database. When a PeLAR was located, all study procedures already performed and yet to be completed were explained, and their informed consent for continued participation requested. In addition, the patients were provided with the ACIT II Protocol v2.0 13 17.06.2010 (*Appendix 2*) and informed that they have the right to deny continued participation at any point in time. The enrolled patient was examined daily to determine if and when he/she was able to consent for himself/herself even if surrogate consent had already been obtained.

While the duration of unconsciousness for trauma patients is extremely very variable (hours to weeks), the majority regained consciousness within two to 10 days. At this time, the trial and all study procedures—performed and yet to be completed—were explained to the patient in detail. As before, patients were offered the opportunity to give informed consent to continue participation or to withdraw from the study entirely.

A quarterly report was sent to the LREC regarding the consent process. This report included the number of subjects enrolled in the study, the number of subjects for whom consent was obtained prior to entry, the number of subjects for whom consent was waived, the number of subjects or PeLARs who later refused or agreed to continue in the study, and on going study results available.

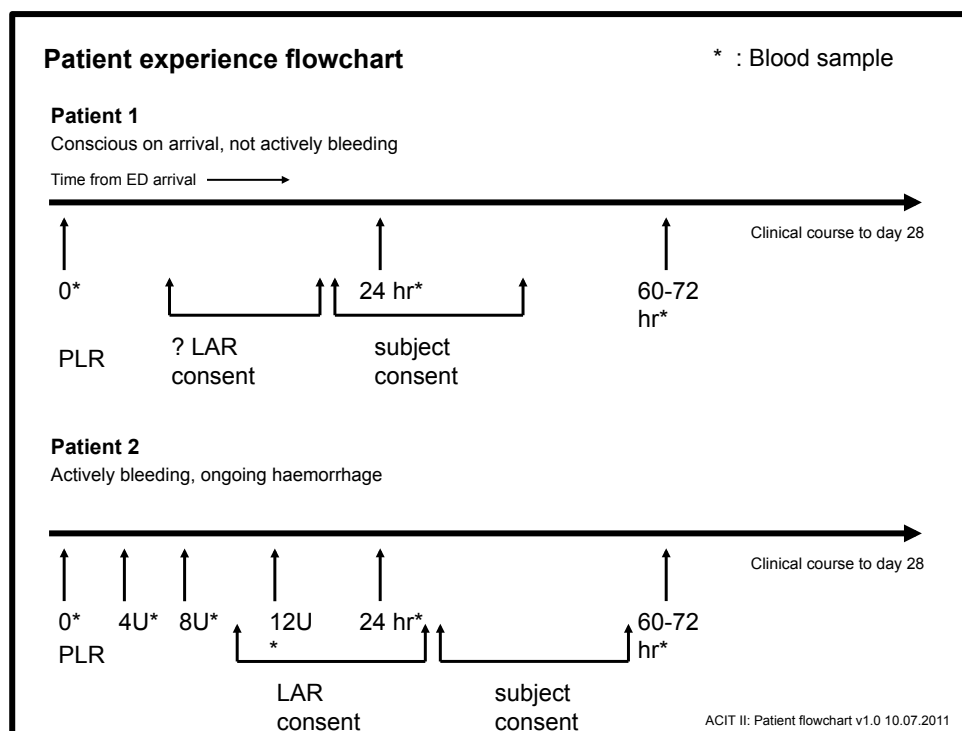
## **2.4 Blood collection**

Thirty ml of blood was drawn from either the femoral vein or the antecubital fossa for each patient enrolled. This baseline sample was the first sample collected within 20 minutes of the patient's arrival in the ED. Subsequently, further follow-up samples of 30 ml were obtained at h 24 and 72: these were successively analysed and then banked for future data analysis. A flow chart detailing the standard operating procedure (SOP) was made available within the ED for ease of reference (*Figure 2.1*).



**Figure 2.1. The ACIT II ED sample SOP. Located within the ED.**

Those patients that required a blood transfusion had a further 30 ml of blood collected after the 4th, 8th and 12th units of PRBC transfusions. This subset of trauma patients is referred to as “bleeding” patients. *Figure 2.2* outlines the patient journey.



**Figure 2.2. Summary of patient experience through the ACIT II study process.** *Patient 1: no active bleeding. Patient 2: active bleeding.*

At the baseline, all patients had an arterial sample taken for blood gas analysis, including the measurement of BD and lactate. A standard trauma panel of blood tests were obtained at each point of time for routine clinical use. These included: FBC, PT, aPTT and fibrinogen, and U&E. The blood samples required specifically for research use were collected in specific vacutainers (Becton Dickinson, Plymouth, UK) (*Table 2.1*). After collection, all samples were processed in the Trauma Clinical Academic Unit (TCAU) laboratory and frozen at -80°C within two hours of the blood being drawn.

Type	Blood volume	Preservative	Intended use
<b>Coagulation</b>	2.7 ml	0.109 M buffered sodium citrate (3.2%)	ROTEM
<b>Coagulation</b>	2.7 ml	0.109 M buffered sodium citrate (3.2%)	Research coagulation marker assays. Inflammatory marker assays. Genomics.
<b>Proteomics (P100)</b>	6.5 ml	Protease Inhibitor	Activated Protein C assay. Assays of unstable proteases.
<b>RNA (PAXgene)</b>	2.5 ml	RNA stabiliser	RNA analysis.

**Table 2.1. Blood sample collection tubes.**

Near patient testing was carried out using facilities in the Trauma Sciences Group laboratory at the TCAU (*Figure 2.3*). This laboratory is located within the hospital in close proximity to the ED to ensure quick processing of blood samples for patients that need ongoing resuscitation with blood component therapy. A research assistant provided help with blood sample processing and data capture. During the study period, two other research fellows were recruited to increase patient enrolment. To ensure quality control throughout the study period, all researchers and assistants performing ROTEM analyses and blood processing undertook a period of training with ongoing supervision. During the study period, the ACIT II protocol was introduced to two other sites and involved utilising the identical inclusion criteria and blood processing techniques as detailed below. These

external sites were the John Radcliffe Hospital, Oxford, United Kingdom and Oslo University Hospital Ullevaal, Oslo, Norway. Along side the clinical trials coordinator I was responsible for implementation of the ACIT II protocol and auditing the adherence to the ACIT II inclusion criteria. Both of these recruitment sites were equivalent Level 1 Trauma centres. All study patients across the sites were treated with 24/7 access to damage-control surgery with anatomic control of hemorrhage and therefore completion of transfusion was rapid and typically occurred within the first 2 hours to 3 hours following admission. However due to the lack of permanent, full time research staff resulting in recruitment time constraints these sites only contributed to 5% of the total study participants (for more details, see Chapters Four Five and Six).



**Figure 2.3. TCAU blood sample processing facilities at the Trauma Sciences Group laboratory.**

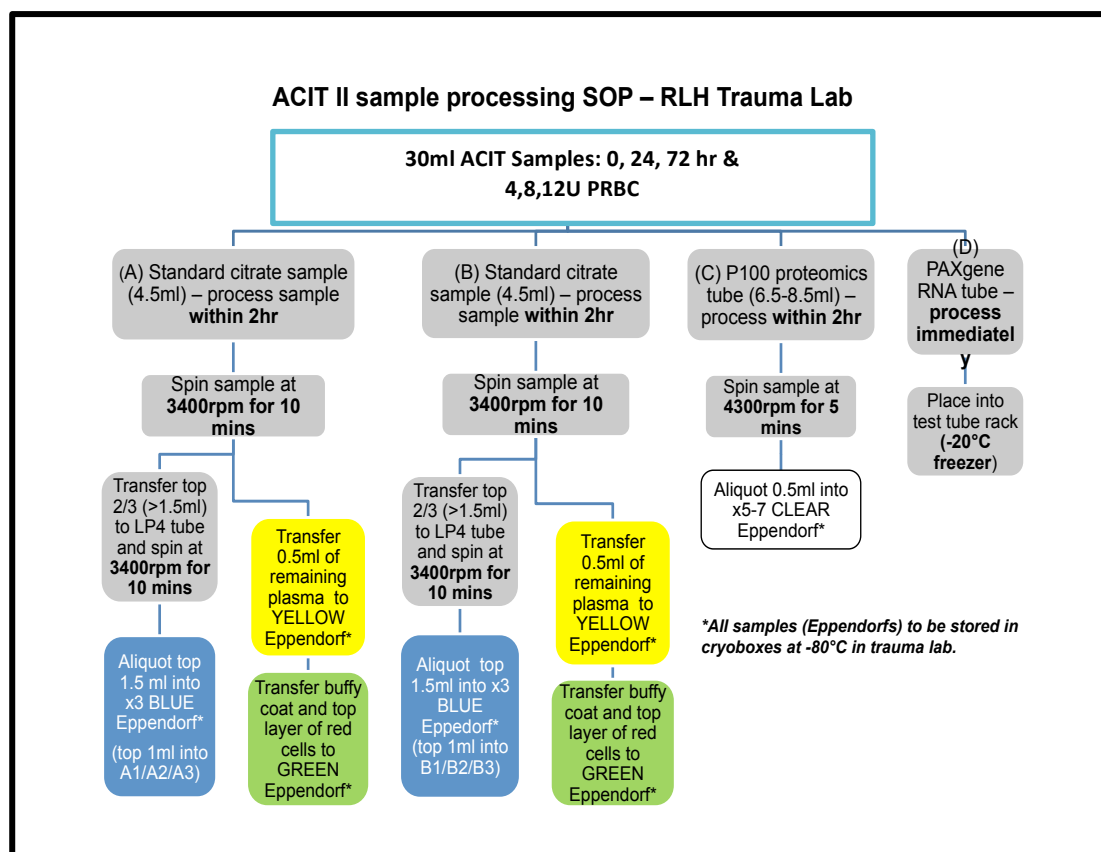
### **2.4.1 Samplings materials**

Throughout the study period, sample tubes and documentation were included in the ACIT II trial packs and available in ED clinical trials area. Each study trial pack contained:

- Eight sets of vacutainer tubes (hours: 0, 24 and 60–72), labelled with the ACIT II barcode.
- Sample processing instructions.
- The flow chart shown in *Figure 2.1*.
- Cryotubes for aliquoting of samples, labelled with the ACIT II barcode.

## 2.4.2 Coagulation biomarkers

At each time point, the citrated 4.5 ml tubes were centrifuged at 1760g for 10 min in a Clinispin horizon 853VES laboratory centrifuge (Woodley Equipment Company Ltd, Bolton, UK), located within our TCAU laboratory. The top two thirds of single-spun plasma was removed and centrifuged again at 1,760 g for a further 10 min. A 0.5 ml aliquot of single-spun plasma was removed after the first centrifugation (i.e. the bottom one third of the plasma) and stored in a 0.6 ml Cltiklok microcentrifuge tube (Simport, Jencons, UK). The remaining single-spun plasma, also known as the “buffy coat”, and the top layer of red cells were removed and stored in a 1.5 ml Fisherbrand microcentrifuge tube (Fisher Scientific, UK). The top two thirds of double-spun plasma was stored in three 0.5 ml aliquots in separate 0.6 ml Cltiklok microcentrifuge tubes (Simport, Jencons, UK). All of the blood sample aliquots were individually barcoded and stored in grid-labelled cardboard cryoboxes at -80°C. Each of the samples were catalogued on an Excel spreadsheet. *Figure 2.4* outlines the blood collection process.



**Figure 2.4. ACIT II sample processing SOP chart.**

## 2.5 Blood sample analysis

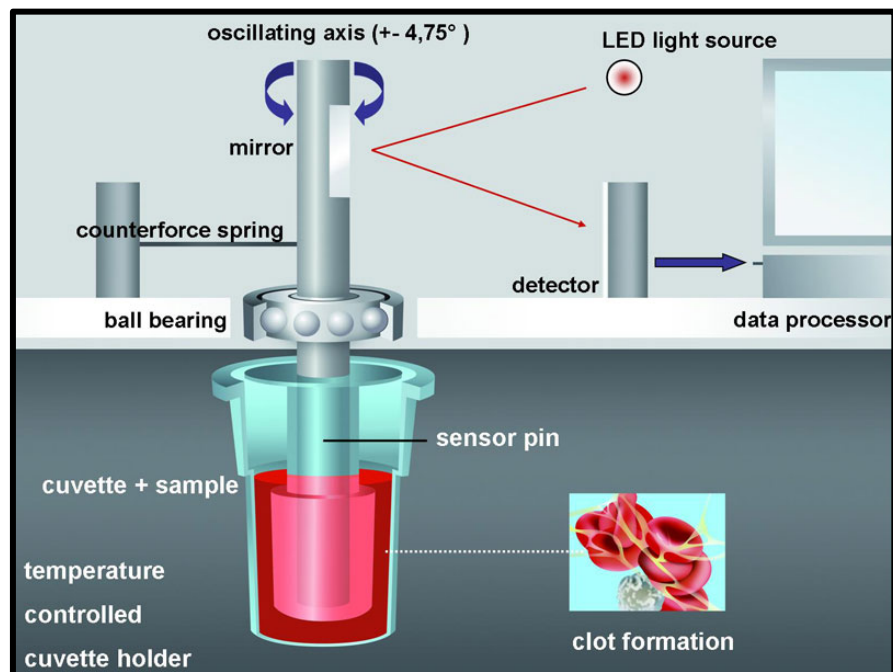
### 2.5.1 ROTEM analysis

The ROTEM parameters analysed are those detailed in *Table 1.2*. All of the pipetting steps and the mixing of reagents with blood were standardised as per the ROTEM automated electronic pipette program. ROTEM analysis was performed at 37°C on three channels (INTEM, EXTEM, FIBTEM). The ROTEM Delta machines were located in the TCAU laboratory. It was possible to run three samples simultaneously.

The vacutainer containing blood is placed in the heating well for 10 min prior to analysis in order to incubate sample at 37°C (*Figure 2.5*). After 10 mins, four plastic cups and pins



were positioned onto each of the test blocks and rotating pins. The individual ROTEM reagents were removed from the fridge for 10 mins prior to use: STAR-TEM (recalcitrant); EXTEM (thromboplastin); and INTEM (ellagic acid). The following reagent combinations were inserted into the appropriate well followed by 300µl blood for each individual test, and were analysed for 60 mins. EXTEM test: 20ng reagent combination; and INTEM test: 20ng reagent combinations .



**Figure 2.5. ROTEM detection.** *A whole blood sample is placed into a cuvette and a cylindrical pin is immersed. Between the pin and cuvette, there is a gap of one mm, bridged by the blood. The pin is rotated by a spring, moving to the right and the left. As long as the blood is liquid, the movement is unrestricted. When blood starts clotting, the clot increasingly restricts the rotation of the pin with rising clot firmness. This kinetic is mechanically detected and calculated by an integrated computer to the typical curves (TEMogram) and numerical parameters. (Taken with permission from ROTEM® 2014 Tem International, GmbH.)*

Precision	CT	CFT	Alpha angle	CA5	MCF
Intra-assay	6	8	1	2	3
Inter-instrument	7–13	5–8	2–3	2–3	1–3

**Table 2.2. ROTEM repeatability and reproducibility.** (*Coefficient of variation %.* *Provided by ROTEM GB.*)

## 2.5.2 Definition of coagulopathy

Throughout this thesis, I have defined coagulopathy on the ROTEM as a five-min EXTEM clot amplitude (CA) of  $\leq 35$  mm (75, 124). It has been previously shown that this definition can accurately identify ATC and predicts the need for MT (MT is defined as  $\geq 10$  units PRBC in 24 h) (124). Unless otherwise stated, this definition of ATC has been used.

## 2.5.3 Sysmex – an automated coagulation analyser

### Factors II, V, VII, VIII, IX, X, XI von Willebrand Factor (vWF) antigen

Fresh blood samples were prepared to extract double-spun plasma for analysis (*Figure 2.3*) and PT, PTT and fibrinogen levels (Clauss method) were measured by the laboratory staff at the RLH's central laboratory. The frozen aliquots were thawed using a water bath at  $37^{\circ}\text{C}$  prior to analysis. Normal reference ranges for clotting times and coagulation proteases are detailed in *Table 2.3*. PTr was calculated as observed: PT divided by mean control PT.

Parameter	Normal range	Assay method	Intra-assay variability	Inter-assay variability
<b>PT</b>	9.4–12.4 s	Clotting	1.3%	2.0%
<b>INR</b>	0.9–1.1	Clotting	1.3%	2.0%
<b>aPTT</b>	21–31 s	Clotting	4%	4%
<b>Fibrinogen</b>	1.50–4.5g/L	Clauss	5.9%	2.3%
<b>Factor II</b>	58–155	Clotting multi-dilution analysis	by 2%	7%
<b>Factor V</b>	60–150	Clotting multi-dilution analysis	by 2%	7%
<b>Factor VII</b>	50–150	Clotting multi-dilution analysis	by 2%	7%
<b>Factor VIII</b>	52–153	Clotting multi-dilution analysis	by 2%	7%
<b>Factor IX</b>	52–156	Clotting multi-dilution analysis	by 2%	7%
<b>Factor X</b>	50–150	Clotting multi-dilution analysis	by 2%	7%
<b>Factor XI</b>	50–150	Clotting multi-dilution analysis	by 2%	7%
<b>vW:Ag</b>	50–155	Clotting multi-dilution analysis	by 1.4%	0.9%

**Table 2.3. Method of coagulation assays and coagulation laboratory reference ranges.**

### 2.5.4 Procedure for vW:Ag assay

The blood samples are deemed stable for four hours if left undisturbed at room temperature. Prior to testing, each of the blood is double-spun (centrifuged at 2500g for 10 min) and the plasma removed into a plastic tube and recentrifuged at 2,500g for 10 minutes, to generate platelet-poor plasma (<5,000/ $\mu$ l). In instances where testing was delayed for longer than four hours from time of collection, centrifuged samples had the plasma layer transferred to a plastic tube and flash-frozen at <-80°C. Just prior to testing, the frozen aliquot is rapidly

thawed at 37°C and testing performed within two hours of thawing. The equipment required for analysis is detailed below.

**Equipment required:**

- Sysmex CS2100i analyser.
- Sysmex sample racks.
- Cuvettes.
- Two ml sample cups
- Finnpipette 200–1,000µl.
- Finnpipette tips 200–1,000µl.

**Reagents and preparation**

Pre-prepared Siemens vWF:Ag kits were available (reference number OPAB03). Each of the kits contained:

- Buffer: 4x5 ml bottle of Glycine buffer. Ready for use. Stable for 15 days at 2–8°C.
- Reagent diluent: 4x4 ml Glycine diluent.
- Reagent: 4x2 ml bottles of a suspension of polystyrene micro-particles coated with anti-human vWF antibodies.

**Procedure**

The reagents and control were reconstituted at the same time. The buffer was placed in a free position within the reagent ring in the analyser. In this position, the barcode can be read and the reagent recognised by the analyser. Next, the contents of one vial of the

reagent were added and the sequence started. When the analysis cycle was complete, the results of the QC run were checked. If the results were within the acceptable range, the patient's samples were run. However, if the QC was out of range, the assay had to be recalibrated.

The test for any patient whose result was less than 15 iu/dl was automatically repeated. The sample for any patient whose result was greater than 180 iu/dl was automatically rediluted to give a more accurate result.

### **2.5.5 Procedure for coagulation factors**

The blood sample was collected as outlined above. Prior to testing, blood was centrifuged at 2000g for 10 min to generate platelet-poor plasma (<10,000/ $\mu$ l). Again, if testing was to be delayed for longer than four hours from the time of collection, it was necessary to freeze the plasma by transferring the plasma layer to a plastic tube and storing it at <-80°C. Just prior to testing, the frozen aliquot is rapidly thawed at 37°C and testing performed within two h of thawing.

#### **Equipment required:**

- Sysmex CS2100i analyser.
- Sysmex sample racks.
- Sysmex reagent inserts.
- Two ml sample cups.
- Four ml sample cups.
- Finnpiptette 200–1,000 $\mu$ l.
- Finnpiptette tips 200–1,000 $\mu$ l.
- Finnpiptette 1–5 ml.
- Finnpiptette tips 1–5 ml.

The reagents and controls were respectively reconstituted as detailed above. Once the reagents and control were reconstituted, the required factor deficient plasma was decanted into a two ml sample cup (if one reagent bottle was reconstituted) or a four ml sample cup (if two reagent bottles were reconstituted). The sample cups were then loaded into analyser in four ml reagent inserts with their assigned barcode labels. From the main menu, it was possible to select '**Order**', then '**Switch Order**' and then '**Holder QC Order**', depending on the order of the samples. Next, the required factor deficient assay for each QC was requested and then '**Start**' and '**Start Measurement**' was pressed. When the analysis was complete, the results of the QC run were rechecked. As stated above, if the results were within the acceptable range, the patient's samples could be run.

## 2.6 Data collection

At each blood sample time point, heart rate, SBP, respiratory rate, Glasgow coma scale (GCS), temperature and the time the blood was drawn were recorded. Patient demographics, mechanisms of injury, injury type and severity (ISS) were collated from the RLH trauma registry. Each injury was scored and classified according to the AIS to provide a composite anatomical ISS (Baker et al., 1974).

Specific outcomes include hospital length of stay (LOS), critical care days and ventilator-free days. Total intravenous fluid and transfusion requirements from injury to 12 h and from 12 h to 24 h were collated. Times of all transfusions (crystalloid fluids, colloids, PRBCs, FFP, CRYO, platelet concentrates) in the first 24 h were recorded to calculate ratio of blood product replacement.

## **2.7 Statistical analysis**

Statistical analysis was performed with GraphPad Prism 4.0 (GraphPad Software Inc., La Jolla CA, US) and Microsoft Excel 2010 (Microsoft Inc., Redmond WA, US). Normal quantile-quantile plots were used to test for normal distribution. Parametric data are expressed as mean  $\pm$  standard deviation (SD) or 95% confidence intervals. Non-parametric data are reported as median (interquartile range). A two-group analysis was performed with a two-tailed unequal variance Student's t test for parametric data and Mann–Whitney U test for non-parametric data. ROTEM changes over time were analysed using a two-way analysis of variance (ANOVA). Proportions were assessed using Fisher's exact test and the chi-squared test for trend. A p value of 0.05 was chosen to represent statistical significance.

## CHAPTER THREE

# **The effect of a major haemorrhage protocol at a level 1 trauma centre**



### 3.1 Introduction

A growing body of literature points to a paradigm shift from reactionary trauma protocols towards more focused, goal-directed MHP.

Death due to traumatic injury is the leading cause of lost life years throughout the world (166). Severe haemorrhage accounts for almost 50% of deaths and the majority of these occur within the first 24 h of injury (123). MT among severely injured trauma patients is not uncommon. Studies have shown that 15% of patients in major trauma centres receive a MT and over 25% of these will die, most within six hours of injury (167). Furthermore, patients who survive a MT have an increased incidence of sepsis, multi-organ failure, longer hospital stays and higher healthcare costs (162, 163).

The concept of MT was originally introduced to highlight the complications that result from large volume PRBC infusion—principally, late dilutional coagulopathy (168). Traditionally, resuscitation has been initiated with large volumes of crystalloid (ATLS), then accompanied by PRBC therapy based on laboratory values and at the discretion of the TTL, ED physicians and surgical teams. MTPs, therefore, initially delivered PRBCs and provided relatively small volumes of blood component therapy (FFP, PLTs and CRYO) only after sufficient units of PRBCs had been transfused to cause a dilutional coagulation dysfunction (167, 169). It is for this reason that MTPs may therefore be considered more reactionary to large volume blood product replacement and crystalloid therapy (101) in comparison to MHPs. The discovery of ATC (34) suggests that this coagulopathy is rapidly established after injury and is best treated much earlier in the clinical course (167, 170). There is no absolute consensus in the definition of MT, although the general clinical population have assigned an arbitrary value of >10 U PRBCs within 24 h. Furthermore, the clinical identification of patients who require more than a set number of PRBC units is

difficult (167). Additionally, traditional MTPs have been shown to be ineffective in treating ATC and reducing blood transfusion requirements (47), as this treatment strategy tackles coagulopathy late in the clinical setting, after the patient is stabilised and the acute resuscitation complete. It is for these reasons that MT may therefore be an outdated concept in the management of trauma haemorrhage. In recent times, newer strategies that directly target ATC, such as DCR (39), require protocols that rapidly identify bleeding patients and deliver high-dose coagulation therapy. These MHPs must also avoid over-provision, over-transfusion or waste of blood products, a significant issue with existing transfusion therapy in trauma (162, 163).

### **3.2 Aims**

The hypothesis of this study is MHPs improve blood product administration and reduces waste compared to traditional MTPs.

Firstly, I aimed to ascertain if an MHP could appropriately identify patients who were bleeding and would need a  $\geq 10$  U PRBC transfusion. Second, I wished to determine whether the MHP activation criteria could be correctly initiated by the TTL. Third, I wanted to ascertain whether there was any improvement in the administration of blood components and fourth, if there was a reduction in the waste of blood components. Finally, I wished to determine whether there was an improvement in patient outcomes with an MHP. I conducted a retrospective one-year before and after the cohort study of all adult trauma patients after implementation of an MHP at the RLH.

### 3.3 Methods

#### 3.3.1 Study design

In September 2008, the RLH, a major trauma centre, switched from a standard MTP to a newer MHP. The previous MTP was broadly based on the British Committee for Standards in Haematology guidelines (*Figure 3.1*) (81, 84, 171). There were no predefined triggering criteria and the MT Clotting Pack (standard MTP) of products could only be requested on the instructions of a senior doctor (ED registrar or consultant) who was directly in charge of the patient (not the pre-hospital team). Conversely, the new MHP was developed and driven by a multidisciplinary team from the RLH that included pre-hospital and emergency physicians, trauma surgeons, haematologists and the blood transfusion laboratory (*Figure 3.2*). This newer MHP has strict activation criteria and can be initiated by pre-hospital teams, by the ED or by the operating room staff. When activated, transfusion begins with PRBCs that are held in a blood fridge within the ED, close to the trauma patient. The first major haemorrhage pack from the blood bank (Pack A) contains six PRBCs and four FFP units. If bleeding persists, a Pack B—containing six PRBCs, four FFP, one pool of platelets and two pools of CRYO—is ordered from the blood bank that is located adjacent to the ED. Pack B is issued repeatedly until the bleeding is controlled. Antifibrinolytics, recombinant factor VIIa, or other procoagulants were not routinely used during the period of study.

### Figure 1: Massive Transfusion Protocol Guidelines

Clotting Pack 4 units Fresh Frozen Plasma, 1 unit Platelets, 5 units Cryoprecipitate

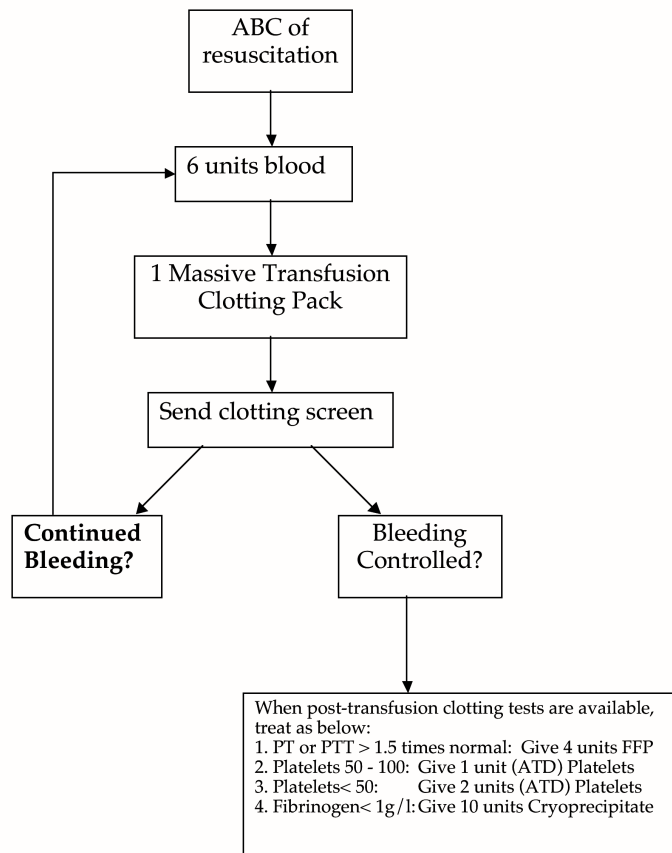


Figure 3.1. The RLH's massive transfusion protocol. (Pre-September, 2008.)

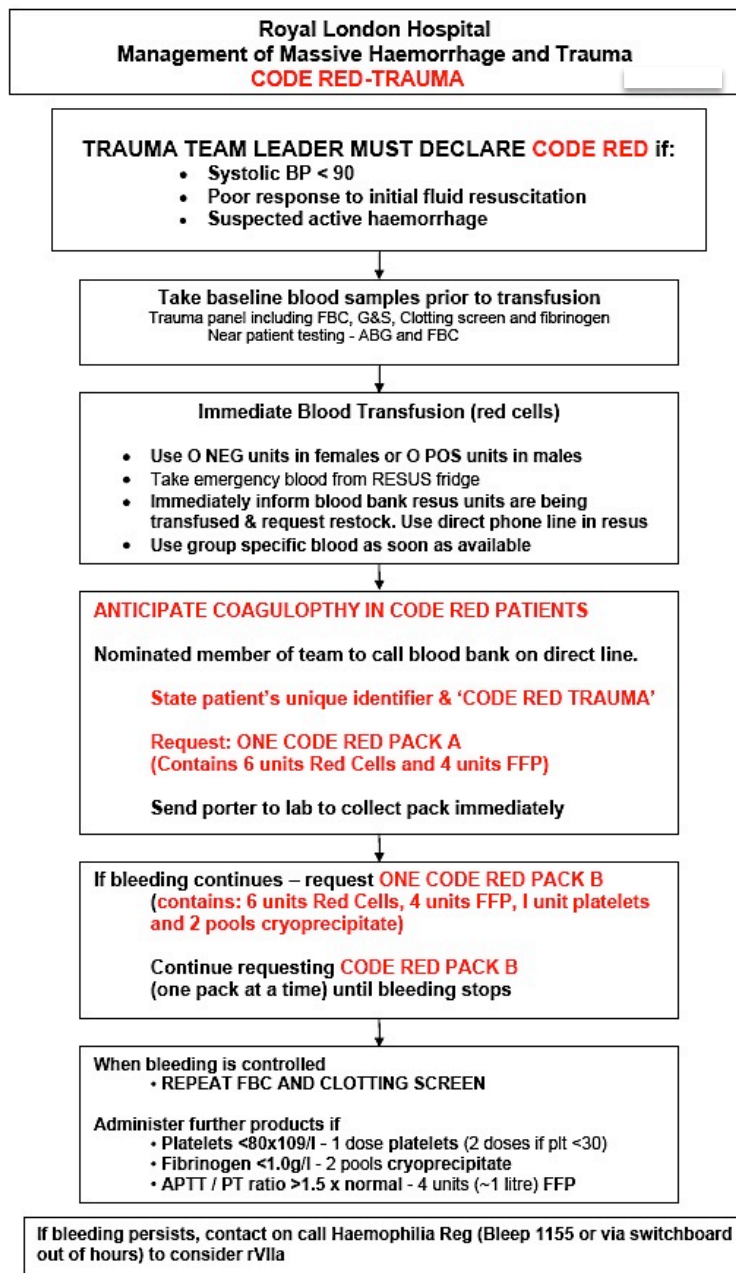


Figure 3.2. The RLH's major haemorrhage protocol. (*Code Red Policy.*)

### **3.3.2 Participants**

Two consecutive 12-month time periods before (MTP: September 2007–August 2008) and after (MHP: September 2008–August 2009) the new protocol implementation were analysed. In order to include all the patients that would potentially receive a MT, patients that were transfused greater than 4 U of PRBCs pre- and post implementation of an MHP were initially included in this study. Although this chapter is primarily interested in the efficacy of the protocols for patients receiving a MT (10 or more units of PRBCs) (101), this study included the larger cohort to allow analyses of any effect of the MHP on the reduction of PRBC transfusions.

### **3.3.3 Data collection**

Patient information was collected using the RLH trauma registry. Demographic and clinical data were collected prospectively, including mechanism of injury, physiological observations, ISS, ICU admission and length of stay. Injury severity was defined using the ISS (8).

The volume of blood components (PRBCs, PLTs, CRYO and FFP) issued, transfused, returned to stock and wasted within the first 24 hours was gathered retrospectively from the hospital transfusion database, ED and ICU charts. Wasted blood products were defined as those that were issued by the transfusion laboratory and not transfused nor returned to stock. MT was defined as receiving 10 or more units of PRBCs in the first 24 h (45). Shock was defined as a SBP<90 mmHg. Clinical outcomes were recorded in hospital death, ICU and hospital stays.

### **3.3.4 Data analysis**

Statistical analysis was performed using GraphPad PRISM v5 (GraphPad Software Inc, San Diego, CA, US) and Microsoft Excel 2007 (Microsoft, Inc., Redmond, WA, US). The groups were tested for normality using normal quantile–quantile plots. Proportions were analysed using the chi-squared test and Students t test for continuous variables.

## **3.4 Results**

In the two-year study period, there were a total of 2,986 adult trauma team activations and an 8% increase in overall activity in the second year (*Table 3.1*). Patients in the MHP group were more severely injured, but a similar proportion arrived in shock and received four or more units of PRBC transfusions. Overall, 3% (n=) of adult trauma calls received  $\geq 10$  units PRBCs in the MTP group and 4% received the same in the MHP group. Patients arriving in shock were more likely to receive a MT in the MHP group compared to the MTP group (74% vs 43%,  $p < 0.001$ ). Clinical characteristics of the MT patients are detailed in *Table 3.1*.

	September 2007–2008	September 2008–2009	P value
<b>Number</b>	1,438	1,548	
<b>Median age</b>	32 (23–45)	30 (22–44)	0.65 <sup>1</sup>
<b>Male</b>	1,183 (82%)	1,290 (83%)	0.85 <sup>2</sup>
<b>Admitted</b>	945 (66%)	1,079 (69%)	0.65 <sup>2</sup>
<b>ISS&gt;15</b>	353 (25%)	430 (30%)	0.43 <sup>2</sup>
<b>SBP&lt;90 mmHg</b>	93 (6%)	80 (5%)	0.76 <sup>2</sup>
<b>Transfused≥4 U PRBC</b>	100 (8%)	145 (9%)	0.80 <sup>2</sup>
<b>ISS&gt;15</b>	82 (82%)	119 (82%)	1.00 <sup>2</sup>
<b>SBP&lt;90 mmHg</b>	32 (32%)	41 (28%)	0.54 <sup>2</sup>
<b>Median PTr</b>	1.2 (1.1–1.4)	1.2 (1.1–1.3)	0.43 <sup>2</sup>
<b>PTr&gt;1.2</b>	52 (52)	83 (57)	0.48 <sup>2</sup>
<b>Hospital LOS</b>	17 (38–64)	7 (16–29)***	0.0006 <sup>1</sup>
<b>ICU LOS</b>	9 (1–19)	5 (1–15)	0.60 <sup>1</sup>
<b>% Admitted to ICU</b>	<b>67 (67%)</b>	<b>84 (58%)</b>	<b>0.19<sup>2</sup></b>

**Table 3.1. Clinical characteristics of all trauma patients.**

*Clinical characteristics of all trauma patients from September 2007 to September 2009. Values are given as numbers (%) or medians (interquartile range). SBP: Systolic Blood Pressure; ISS: Injury Severity Score; PTr: Prothrombin ratio; PRBCs: Packed Red Cells; FFP: Fresh Frozen Plasma; PLT: Platelets; CRYO: Cryoprecipitate; LOS: Length of Stay; \*  $p<0.05$ . \*\*  $p<0.01$ , \*\*\* $p<0.001$ . <sup>1</sup>:Students T Test. <sup>2</sup>:Chi-squared test/Fisher's exact test.*

Next, I examined the efficiency of the activation criteria in identifying those patients who would receive a MT (Table 3.2). When analysing the number of the patients who were transfused four or more units of PRBCs, 50 patients (54%) met the MHP activation criteria and went on to received  $\geq 10$  U PRBCs. Twenty-five patients who, in spite of not meeting the MHP criteria (48% of 52), went on to receive a MT. On average, patients that met the activation criteria went on to receive nine PRBC units vs five PRBC units for those that did not fulfil the criteria for activation ( $p<0.01$ ). The MHP criteria had 67% sensitivity and 33% specificity for predicting the need for  $\geq 10$  units PRBCs.



	Transfused 4–9 U PRBC	Transfused 10+ U PRBC
<b>Met MHP criteria</b>	43	50
<b>Did not meet the MHP criteria</b>	27	25

**Table 3.2. Sensitivity and specificity of MHPs.**

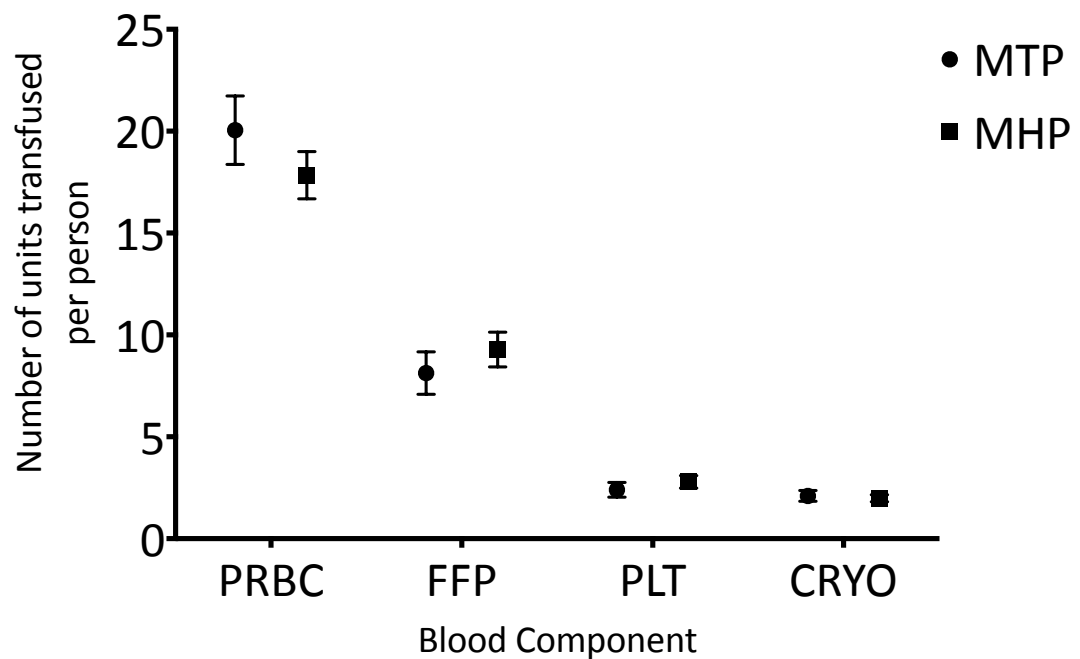
I next examined whether the MHP was correctly initiated by the TTLs according to the activation criteria (*Table 3.3*). Seventy-five patients (81%) who met the criteria had the MHP correctly activated. On average, these 75 patients were transfused nine PRBCs and the 18 that were not activated (but met MHP criteria) received an average of eight PRBCs. The team leaders correctly identified 47 (90%) of the patients that did not meet the MHP criteria. In five patients (10%), the MHP was activated despite not fulfilling the criteria and none of these went on to require a MT (*Table 3.4*).

	Met MHP criteria	Did not meet MHP criteria
<b>Activated by TTL</b>	75	5
<b>Not activated by TTL</b>	18	47

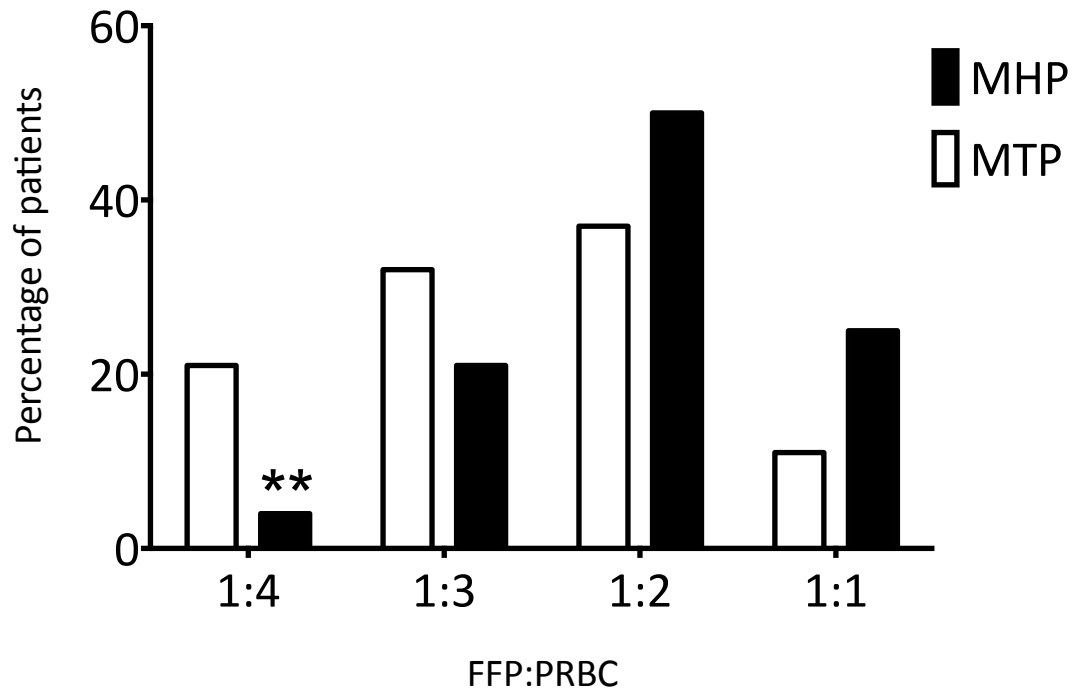
**Table 3.3. TTL's ability to correctly initiate MHP.**

To determine whether there was an improvement in blood product administration, the volume of blood components transfused per person was analysed (*Figure 3.3*). After implementation, there was a two-unit average reduction in PRBC transfusion per patient.

MHP patients received an improved ratio of both FFP:PRBC and CRYO:PRBC (*Table 3.4*). There was a significant increase in FFP transfused for the MHP cohort with an average ratio of 1:2 vs 1:3 for MTP patients ( $p<0.01$ ). Similarly, the CRYO:PRBC ratio increased from 1:10 to 1:7 ( $p<0.05$ ) in the MHP cohort. Fewer patients received inadequate doses of FFP, with the number of patients that were transfused with an FFP:PRBC ratio of 1:4 was reduced from 20% to 3% (*Figure 3.4*,  $p<0.01$ ). The delivery of platelets also improved, with a 14% increase from 72% to 86% in transfusion after the implementation of the MHP.

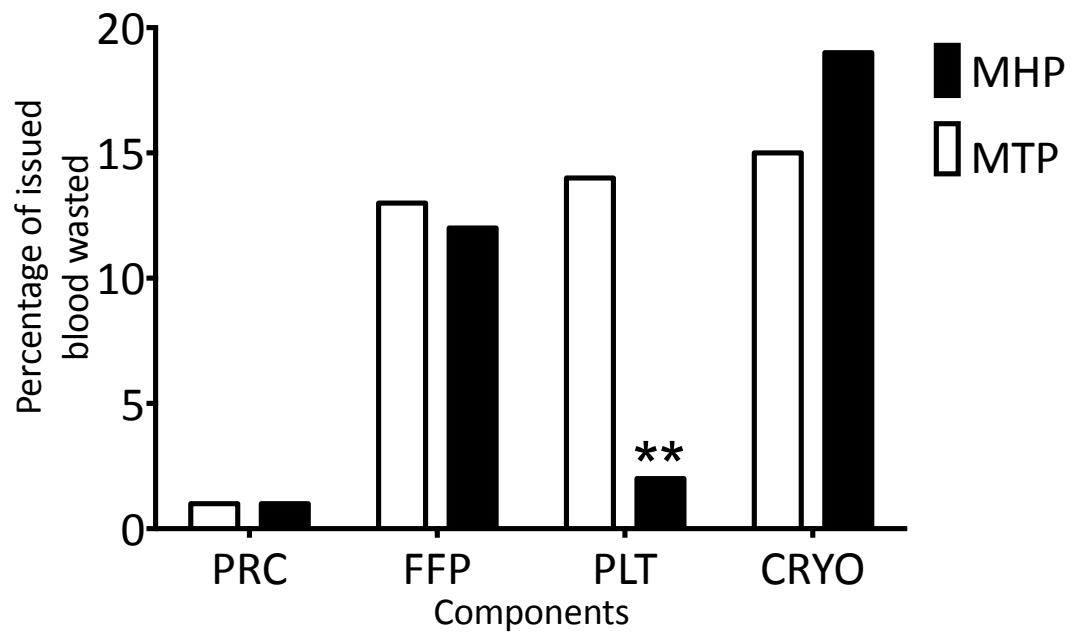


**Figure 3.3. The effect of a MHP on blood component delivery.** *Values are mean ± SEM. Although the transfusion of blood components was similar between the two groups, there was a reduction of 2.2 units of PRBCs and 1.2 pools of FFP transfused.*

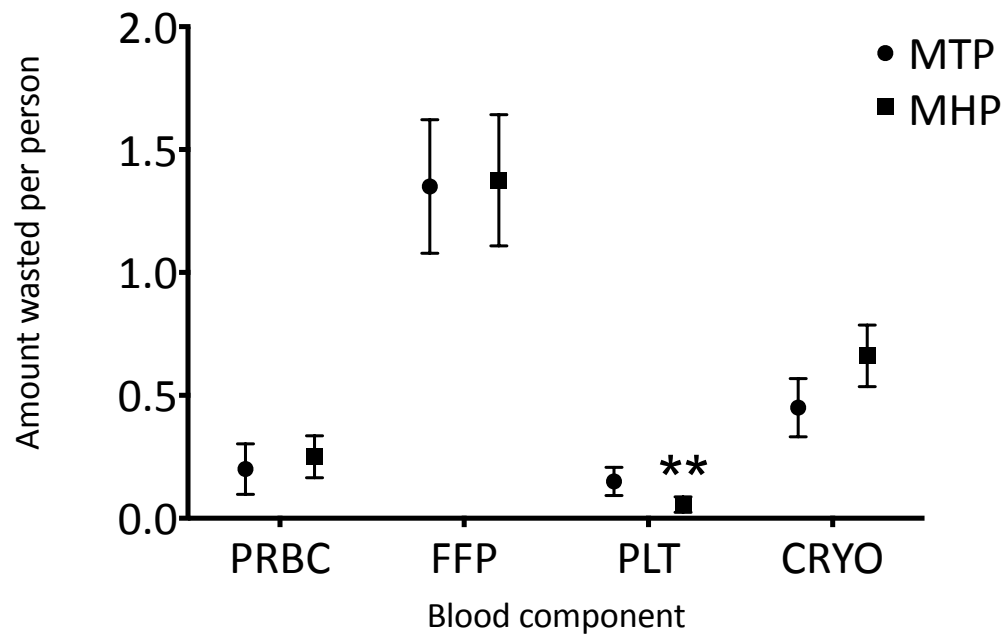


**Figure 3.4. Effect of a MHP on FFP delivery.** *The change in the percentage of patients receiving differing ratios of FFP:PRBCs. The proportion of patients that received FFP:PRBCs of 1:4 was reduced by 17% ( $p<0.01$ ). There is a trend towards transfusing higher ratios of FFP:PRBCs post introduction of the MHP, with over 50% of patients receiving a ratio greater than 1:2.*

I analysed the difference in the waste of blood products after the implementation of an MHP. This data showed a 1% decrease in the total waste of FFP (*Figure 3.4*). Total PLT waste reduced (*Figure 3.5*) was from 16 to 3 pools ( $p<0.01$ ), with no real change in FFP wastage. The greatest reduction in waste *per person* was evident in PLT transfusion (*Figure 3.6*). The waste of PLT decreased from 0.4 pools *per person* to 0.05 pools *per person* ( $p<0.01$ ). Although CRYO waste increased from 0.45 pools to 0.66 per person ( $p=0.44$ ), this only represented 19 pools out of a total of 199 issued (9.5%).



**Figure 3.5. Effect of a MHP on blood component waste.** *This refers to the total amount of blood component wasted as a percentage of that which is issued by the laboratory. Post implementation, there was a 1% reduction in FFP waste and a 12% reduction ( $p<0.01$ ) in PLT waste.*



**Figure 3.6. Effect of a MHP on blood component waste per person.** *Values are mean  $\pm$  SEM showing blood component waste per person. Platelets showed the greatest reduction in waste (0.4 pools pp to 0.05 pools pp,  $p < 0.01$ ).*

Finally, I wished to determine if there was a difference in outcomes between these two groups. Although there was a similar mortality between the two groups, there was a significant decrease in the median length of stay of survivors from 54 days to 26 days ( $p < 0.05$ ).

Patients	MTP	MHP	P-value
<b>n</b>	40 (3%)	56 (4%)	0.7004 <sup>2</sup>
<b>Median age</b>	37	34	0.3299 <sup>1</sup>
<b>Male</b>	30 (75%)	44 (79%)	0.6145 <sup>2</sup>
<b>Injuries</b>			
<b>Median ISS</b>	32 (9–54)	29 (22–41)	0.7211 <sup>2</sup>
<b>ISS&gt;15</b>	38 (95%)	51 (91%)	0.2676 <sup>2</sup>
<b>Blunt injury</b>	30 (75%)	44 (79%)	0.5015 <sup>2</sup>
<b>Median SBP mmHg</b>	103 (81–122)	101.5 (78–120)	0.8911 <sup>1</sup>
<b>Per cent with SBP≤90 mmHg</b>	48%	55%	0.9809 <sup>2</sup>
<b>Median PTr</b>	1.3 (1.2–1.5)	1.3(1.2–1.5)	0.2926 <sup>1</sup>
<b>% PTr≥1.2</b>	74%	80%	0.3134 <sup>2</sup>
<b>Blood components</b>			
<b>Total PRBCs issued</b>	1119	1371	
<b>Total PRBCs transfused</b>	802 (72%)	999 (73%)	0.5072 <sup>2</sup>
<b>Total PRBCs RTS</b>	309 (27%)	358 (26%)	0.4000 <sup>2</sup>
<b>Total PRBCs wasted</b>	8 (1%)	14 (1%)	0.4166 <sup>2</sup>
<b>PRBC transfused per patient</b>	20.1	17.8	
<b>Total FFP issued</b>	404	628	
<b>Total FFP transfused</b>	325 (80%)	520 (83%)	0.1761 <sup>2</sup>
<b>Total FFP RTS</b>	25 (6%)	31 (5%)	0.3863 <sup>2</sup>
<b>Total FFP wasted</b>	54 (14%)	77 (12%)	0.6027 <sup>2</sup>
<b>FFP transfused per patient</b>	8	9	
<b>FFP:PRBCs</b>	1:3	1:2**	0.003 <sup>1</sup>
<b>Total PLT issued</b>	116	128	
<b>Total PLT transfused</b>	84 (72%)	111 (87%)**	0.0053 <sup>2</sup>
<b>Total PLT RTS</b>	16 (14%)	14 (11%)	0.4975 <sup>2</sup>
<b>Total PLT wasted</b>	16 (14%)	3 (2%)*	0.0009 <sup>2</sup>
<b>PLT transfused per patient</b>	2	2	
<b>PLTs:PRBCs</b>	1:12	1:10	0.1427 <sup>1</sup>
<b>Total CRYO issued</b>	118	199	
<b>Total CRYO transfused</b>	96 (82%)	156 (78%)	0.5827 <sup>2</sup>
<b>Total CRYO RTS</b>	4 (3%)	6 (4%)	0.7004 <sup>2</sup>
<b>Total CRYO wasted</b>	18 (15%)	37 (18%)	0.4480 <sup>2</sup>
<b>CRYO transfused per patient</b>	2.4	2.8	
<b>CRYO:PRBCs</b>	1:10	1:7*	0.0133 <sup>1</sup>
<b>Outcomes</b>			
<b>Admission to the ICU</b>	25 (63%)	37 (66%)	0.6575 <sup>2</sup>
<b>Median hospital LOS</b>	54 (33–98)	26 (6–74)*	0.0388 <sup>2</sup>
<b>Mortality</b>	<b>22 (55%)</b>	<b>32 (57%)</b>	<b>0.6687</b>

**Table 3.4. Demographics, injury characteristics and 24-h transfusion requirements in patients receiving ≥10 PRBCs.** Values are given as number (%) or median (interquartile range). SBP: Systolic Blood Pressure; ISS: Injury Severity Score; PTr: Prothrombin ratio; PRBCs: Packed Red Blood Cells; FFP: Fresh Frozen Plasma; PLT: Platelets; Cryo: Cryoprecipitate; LOS: Length of Stay; RTS: Returned to Stock; Blood component transfused or wasted given as a percentage of that which is issued. \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ . <sup>1</sup>:Students T Test. <sup>2</sup>:Chi-squared Test. Ratios of blood products are based on averages transfused per patient.

### 3.5 Discussion

This chapter researched the effect of the implementation of an MHP at a major trauma centre. The introduction of the MHP resulted in an improvement in blood product delivery, transfusion practice, reduction in waste and an improvement in outcomes compared to the previous traditional dilution-targeted MTP.

The priorities of shock resuscitation in the past were primarily to support the circulating volume and to reverse metabolic derangements associated with hypovolemic shock (39). Traditionally, this form of resuscitation was initiated with copious volumes of crystalloid that was administered early, later accompanied by PRBC transfusions. Other blood products such as FFP, platelets and CRYO were supplemented based on arbitrary laboratory values and at the discretion of the trauma teams. Coagulopathy was viewed as a secondary by-product of this volume resuscitation, haemodilution, and concurrent hypothermia. In traditional MTPs, clotting therapy was therefore delivered late in the clinical course and treatment further delayed by laboratory-based diagnostics (75) and blood banking logistics. However, evidence shows that these MTPs have minimally effect in trauma haemorrhage (123, 167). DCR addresses the entire lethal triad and, in particular, targets ATC (39). In response, transfusion and trauma services have designed MHPs along the principles of early identification of bleeding patients, anticipation of coagulopathy and the immediate empiric treatment with high-dose blood component therapy.

Trauma patients who met the MHP activation criteria received almost twice as many PRBC units than those that did not (nine vs five units, in this study of all patients who received four or more PRBCs). The sensitivity of the MHP to correctly identify those patients that would go on to require a MT was above 60% and similar to other published series (172, 173). Our group has previously shown that MT cannot be reliably predicted from the clinical variables available on admission (167). However, the goal of the MHP is not to

predict MT but rather to predict patients who are actively bleeding so that coagulopathy treatment can be initiated. Severely injured patients who receive four to nine PRBC units still have over twice the mortality of those receiving less (167), and over 30% of these were coagulopathic on arrival (124). Protocols that aim to identify only those patients who will require a MT will result in delays in identifying life-threatening bleeding, delayed intervention, and worse outcomes (25).

The MHP anticipates coagulopathy and does not require coagulation tests for the provision of blood component therapy. Ideally, FFP, platelets and CRYO should only be given to patients who are coagulopathic and should be specifically guided by the nature of the clotting derangement. Traditional laboratory tests of coagulation take around 60 min to be available to clinicians and point-of-care INR devices may under-read in trauma haemorrhage (75). Thromboelastometry can identify coagulopathic patients within five min, but still requires a blood sample and subsequent provision of blood components (75). It is likely, therefore, that all MHPs will require empiric unguided component therapy in the immediate phases of care, regardless of the availability of newer point-of-care diagnostics.

The MHP provided higher ratios of both FFP and CRYO to the trauma patients. The previous MTP also delivered a relatively high average FFP:PRBC ratio. However, 20% of MTP patients received a FFP:PRBC ratio of 1:4 in the previous MTP, whereas this low ratio provision was almost eliminated by the current MHP. This much narrower dose-range for FFP provision is important in assuring that patients receive the correct therapy. A contemporary aphorism is that protocols should aim for 1:1 FFP:PRBC ratios so that the patients will receive at least a 1:2 ratio. Despite the complexities of the trauma resuscitation environment, trauma pathways should deliver the correct dose of a therapeutic agent to patients. Over a 10 PRBC unit “window”, the MHP aimed to deliver a FFP:PRBC ratio of



1:2. Over 50% of patients received this ratio and there was a narrow range, with 96% of patients receiving between 1:3 and 1:1 ratios.

The MHP reduced waste despite the increased provision of blood products. There is little research in terms of blood component waste in the current literature and very few studies of MTPs address the issue of waste. The most significant reduction in waste was in the transfusion of platelets, with only three pools wasted in the 12 months of the MHP being implemented. Slightly fewer FFP were wasted despite nearly 200 additional units being transfused in the MHP year. There was a modest 3% increase in CRYO waste, which may reflect the increased doses of CRYO administered by the MHP, but correcting this only requires scrutiny and team education. Reduction in waste of blood products rests with team training and highlighting the importance of preservation of blood stocks. MHPs that aim to deliver high volumes of these products to patients must ensure the responsible use of these precious resources.

Massively transfused patients in the MHP group had a 50% reduction in their total hospital stay compared to the MTP patients. Although this is a historic comparison and there were other improvements in service delivery over this time, this is still a dramatic reduction in overall hospital stay. It is possible that improved management of trauma haemorrhage with DCR and the MHP resulted in lower morbidity for these patients, although this data was not collected in this study. I did not see any significant difference in mortality between the two cohorts. The original MTP already delivered a relatively high FFP:PRBC ratio and so would not reflect the benefit of changing to these doses of FFP, as seen in previous studies (160). I also did not see a reduction in the proportion of patients who received a MT or in the average amount of blood products transfused per patient. This finding is consistent with previous studies and it is not entirely clear how increasing FFP or other blood product transfusions lead to improvements in outcomes in these patients. Overall, the MHP was associated with equivalent or improved outcomes for these severely injured patients.

### 3.6 Limitations

There are a number of limitations to my study. Firstly, it is a retrospective review of trauma registry data and compares the MHP with historic controls. Certain assumptions were made when retrospectively ascertaining if the MHP was appropriately activated, especially pertaining to the criterion 'poor response to fluid therapy', which often had to be abstracted from the observation charts. Second, the time of first blood product administration was not recorded in the MTP group and therefore I was unable to determine if the blood components were provided in a more timely fashion compared to the MTP. I also did not notice any reported changes in the coagulation parameters between the two protocols. Although admission coagulation tests were available on all patients, there was no standard time point at which a coagulation test was ordered at the end of the transfusion and thus any improvements in the speed or effectiveness of normalisation of the clotting function could not be analysed. Furthermore, the MHP presented in *Figure 3.1* has since been updated to include other developments, such as the use of tranexamic acid (151, 174). Further studies are planned to assess the impact of this on blood product utilisation and the outcomes. Finally definition of Massive Transfusion was set as those receiving greater than 10u PRBC transfusion within 24hrs (101) at the time of the study this was the conventional definition for MT. However this criteria has subsequently been revised to a more acute definition of at least 5 units in 4 hours (175).

### 3.7 Conclusion

Major haemorrhage protocols are more suited to the current DCR paradigm and offer a number of benefits over tradition protocols targeting MT. MHPs can effectively identify bleeding patients who are likely to develop coagulopathy and benefit from blood component therapy. In conclusion, when combined with a broad staff education

programme, they can significantly improve blood product administration, patient outcomes and reduce waste in trauma haemorrhage. Further work on the effect of MHPs on the coagulation profile during trauma haemorrhage is warranted.

## CHAPTER FOUR

# **Characterisation of damage control**

## **resuscitation use in trauma**

## **haemorrhage**

## **4.1 Introduction**

Uncontrolled haemorrhage remains a leading cause of death in trauma. These severely injured, bleeding trauma patients are delivered to the ED in hypovolaemic shock and suffering from life-threatening TIC. Despite improvements in our understanding of TIC and the adoption of the principles of DCR (39, 130, 176), the mortality in such patients remains high. These strategies aim to improve outcome by promoting haemostasis through the early correction of coagulopathy (134). Numerous civilian and military retrospective studies have shown that the presence of coagulopathy on presentation is associated with worse outcomes in trauma patients with severe blood loss (101, 149). Permissive hypotension and blood component therapy is the mainstay of DCR, with high-dose plasma and platelet transfusions recommended alongside red cell transfusions during the acute haemorrhagic phase. However, the true incidence and evolution of coagulopathy in the acute bleeding phase remains unknown.

Blood components therapy such as platelets (PLT), FFP and CRYO were combined in MHPs (149). These MHPs were designed to identify patients who are actively bleeding, in need of a massive blood transfusion and improve blood component delivery (100), but there is little evidence showing their effect on the coagulation profile during acute active haemorrhage (166). Coagulopathy and hypoperfusion have been shown to correct with DCR, but only at the end of damage control surgery or after admission to critical care, presumably after haemorrhage control has been achieved (177).

## **4.2 Aims**

The overall hypothesis of this study proposes that TIC deteriorates during acute trauma haemorrhage.

The objective of this study is to describe the effect of DCR in the acute phase of trauma haemorrhage.

Specific aims are to characterise the incidence and severity of TIC during the acute phase of DCR; determine whether DCR achieved its stated aims of correction of coagulopathy and restoration of perfusion during trauma haemorrhage; and characterise lactate clearance during haemorrhage.

I conducted a prospective cohort study of bleeding trauma patients presenting directly to three major trauma centres.

## **4.3 Methods**

### **4.3.1 Study design**

All adult trauma patients who met the local criteria for trauma team activation were eligible for enrolment to the prospective ACIT II observational study (outlined in Chapter Two).

The ACIT II study was being conducted at three trauma centres that are members of the International Trauma Research Network (INTRN). These study centres are the RLH, Oxford and Oslo. Subjects were enrolled between June 2008 and June 2013. At this time, the three active study sites had data-sharing protocols and recruitment was limited to times when research personnel were present (08:00 to 22:00). I was the PhD Centre Lead for the ACIT II study and was responsible for ethical approval, data collection, analysis and presentation of the findings, and updating the protocol. Nine-five per cent of the data collected was from the RLH.

The ACIT II protocol blood samples were drawn immediately as the patient arrived at the ED and processed within 20 minutes. Patients that continued to bleed had further samples drawn after four, eight and 12 PRBC units are administered during the acute bleeding phase.

### **4.3.2 Patient selection**

All adult trauma patients requiring trauma team activation were eligible for inclusion. Criteria for trauma team activation were similar across all three sites and included: abnormal physiology (Glasgow Coma Score <14, respiratory rate <10 or <29, SBP <90); anatomical injury (chest trauma with any altered physiology, suspected pelvic fracture, suspected open or depressed skull fracture, amputation proximal to the wrist or ankle, penetrating trauma); and high-energy mechanism of injury (person hit by train, person ejected from vehicle or fatality in same vehicle as occupant, person trapped under vehicle, fall from less than two metres, explosions, industrial accidents).

Exclusion criteria included arrival in the ED more than two h following injury; transfer from another hospital, pregnancy and burns greater than 5% of total body surface area. Patients were retrospectively excluded if they declined to give consent for the research study at any point in time; if they were receiving anticoagulant medications (not including aspirin); and if they had moderate or severe hepatic impairment or a known bleeding diathesis.

This study reports on the subset of ACIT II patients that were bleeding and received four or more units of PRBCs during the acute haemorrhage phase.

The majority of included patients were unable to provide informed consent at the time of enrolment, and consent was therefore obtained from the TTL (a senior ED physician independent to the ACIT II research study) who acted as the patient's legally authorised representative (PrLAR). Written consent from the patient or next of kin (PeLAR) was obtained as soon after enrolment as appropriate. The study was reviewed and approved by the respective National Research Ethics Committees.

### **4.3.3 MHPs**

All hospitals adhered to the principles of DCR: early damage-control surgery and haemostatic resuscitation. We have defined haemostatic resuscitation as: permissive hypotension; limited crystalloid or colloid administration; avoidance of hypothermia, targeting TIC through high-dose blood product administration in the form of FFP, PLTs and CRYO and administration of anti-fibrinolytic agent. (101)

The strict activation criteria for the MHP included signs such as when a patient presented with a SBP of less than 90 mmHg, demonstrated a poor response to initial fluid resuscitation and/or had suspected active haemorrhage (*Chapter 3, Figure 3.2*). The MHP was initiated by pre-hospital teams, or by ED staff. The majority of MHP activations are initiated in the pre-hospital phase by emergency physicians on scene prior to the arrival of the patient in the ED. When activated, transfusion begins with PRBCs that are held in a blood fridge within the ED (immediate availability). For London and Oxford, target ratios for FFP:PRBCs are 2:3 with platelets and CRYO provided after every six units of PRBCs transfused. Delivery is in cooler boxes: pack A contains four units of FFP, while pack B and all subsequent packs contain six units of PRBCs, two pools of CRYO and one pool of platelets. In Oslo, FFP:PRBCs is provided in a 1:1 ratio (Octaplas) with platelets issued with every five units of PRBCs, and monitored according to conventional coagulation tests.



In all centres, resuscitation continues with these multi-component packs until bleeding is controlled.

#### **4.3.4 Sampling technique**

An initial 30 mm research blood sample was drawn along with the standard trauma laboratory blood tests (peripheral blood count, clotting screen, and arterial blood gas) within 20 minutes of the patient's arrival at the ED.

For those patients with *acute* active bleeding, which required immediate transfusion as part of DCR, further blood samples were drawn after the 4th, 8th and 12th unit of PRBCs were transfused and on days one and three of admission. A summary of the SOP for ACIT II study is shown in Flowchart 1, Chapter Two.

#### **4.3.5 Sample analysis**

ROTEM samples were processed using a ROTEM delta instrument (TEM International GmbH, Munich, Germany) at 37°C. The methodology and the parameters of ROTEM have been described previously in Chapter Two (178). Two separate ROTEM assays were performed for each trauma patient. The first, EXTEM, measured tissue factor-initiated clotting and the second is FIBTEM with the addition of cytochalasin D, a platelet inhibitor. All pipetting steps and mixing of the reagents with samples were performed with an automated electronic pipette program. Clotting time, clot amplitude at five minutes (CA5), alpha angle were reported for each sample analysed.

### 4.3.6 Fibrinogen level analysis

Fresh blood samples were prepared to extract double-spun plasma for analysis, and PT, PTT and fibrinogen levels (Clauss method) were measured by the laboratory staff in the central laboratory. Frozen aliquots were thawed using a water bath at 37°C prior to analysis, which used the Sysmex CS2100i automated coagulation analyser. PTr was calculated as observed: PT divided by mean control PT.

### 4.3.7 Definitions

Coagulopathy has been defined on the ROTEM as a five-min EXTEM clot amplitude (CA) less than or equal to 35 ml (14). This has been shown to identify ATC and predicts transfusion requirements (75). From the original 300 patient cohort, I classified abnormal ROTEM parameters as  $\pm 1$  SD from normal ( $PTr < 1.2$ ):  $CT > 94$  seconds,  $CFT > 171$  seconds,  $MCF < 54$  mm,  $\alpha$  angle  $< 65$  degrees. Samples with a prolonged PT ratio ( $> 1.2$ ) were also analysed, as this has previously been shown to be a clinically relevant diagnostic threshold for this measure (65).

Minor injury was defined as any patient who had an ISS (8) less than or equal to four. This cohort of the ACIT II study were used as a baseline comparator, as previously described (154). A lactate level of  $\geq 5.0$  mmol/l was selected to indicate patients with severe haemorrhage (179). I defined normalisation of lactate as levels dropping below an upper limit of normal, i.e. 2.0 mEq/l (179, 180). Completion of PRBC transfusions was used as a surrogate for haemorrhage control.

#### **4.3.8 Data collection**

Data were based prospectively on patient demographics, time of injury, mechanism (blunt or penetrating), pre-hospital fluid administration, time of arrival at the ED, baseline vital signs and total transfusion requirements in the first 12 h of admission. The amount of FFP, PLT and CRYO was recorded after each four units of PRBCs transfused. Ratios of FFP:PRBC, PLT:PRBC and CRYO:PRBC were then calculated for each interval. In addition, the total dose of fibrinogen was calculated using the estimated constituent values above. Coagulation changes for each interval after four units of PRBCs were calculated as: [post-interval sample value - pre-interval sample value].

#### **4.3.9 Statistical analysis**

Statistical analysis was performed using GraphPad PRISM v5 (GraphPad Software Inc, San Diego, CA, US) and Microsoft Excel 2007 (Microsoft, Inc., Redmond, WA, US). Normal quantile–quantile plots were used to test for normal distribution. Parametric data are expressed as mean  $\pm$  95% confidence intervals (CI). Non-parametric data are given as median (interquartile range, IQR). A *p* value of  $<0.05$  was chosen to represent statistical significance throughout.

### **4.4 Results**

There were a total of 810 patients included in the ACIT II study over the study period. Forty-nine patients were retrospectively excluded from the analysis, 28 after personal or legal representative consent was declined following enrolment, and 21 for retrospective exclusion criteria. Of the remaining 761 subjects, 106 were transfused with four or more PRBCs. Clinical characteristics, admission physiology, and laboratory parameters are detailed in *Table 4.1*. The majority of the bleeding patients were severely injured (94%

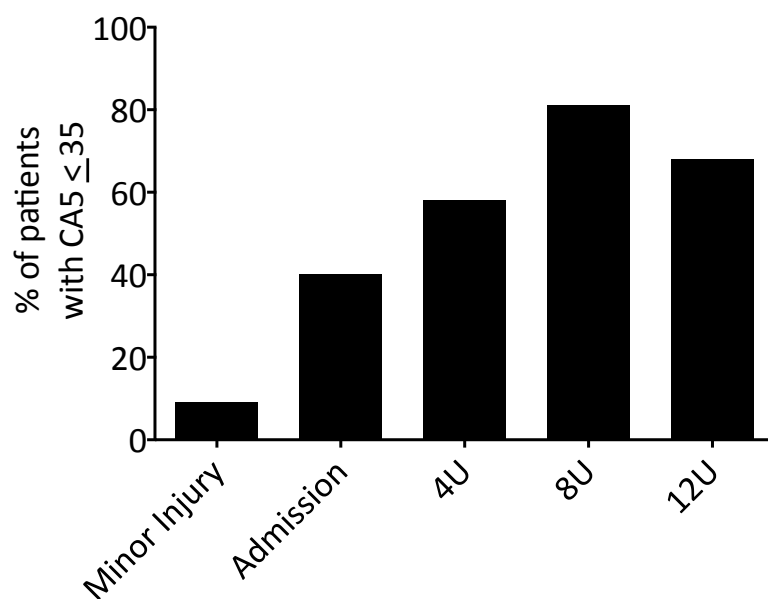
ISS>15) and had sustained blunt injury (87%). Over 40% were coagulopathic on admission by CA5 or PTr. On average, patients required eight units of PRBCs with 38% requiring a MT of 10 or more PRBCs. Early deaths during the bleeding intervals were PRBC 5–8 U: 10 (10%); PRBC 9–12 U: 6 (6%); PRBC 12 U+ to h 12: 6 (6%). The average FFP:PRBC ratio between intervals was PRBCs 0–4 U: 0.5; PRBCs 5–8 U: 0.9, PRBCs 9–12 U: 0.7 and at h 12: 0.7.

<b>Patients</b>	<b>Minor Injury</b>	<b>Bleeding</b>
<b>Number</b>	139	106
<b>Age</b>	33 (22–42)	44 (30–60)*
<b>Male</b>	116 (83%)	75 (76%)
<b>Injury</b>		
ISS	1 (1–4)	34 (25–41)*
ISS>15	0	99 (94%)
Blunt injury	100 (72%)	92 (87%)*
<b>Admission parameters</b>		
SBP<90 mmHg	5 (4%)	41 (46%)*
Base deficit>6 mEq/L	7 (5%)	68 (67%)*
PT (secs)	10.9 (10.5–11.3)	12.9 (11.7–14.6)*
PTr>1.2	2 (2%)	40 (40%)*
CA5 ≤35 mm	12 (9%)	41 (43%)*
<b>Fluid requirements (first 12 h)</b>		
Pre-hospital PRBCs (units)	0 (0)	0 (1–6)*
Pre-hospital Crystalloid (ml)	0 (0–100)	500 (100–2000)*
Crystalloid (ml)	0 (0–800)	2,500 (1300–4000)*
Colloid (ml)	0 (0)	875 (0–1688)*
PRBC (units)	0 (0)	8 (5–13)*
PRBC ≥10units	0 (0)	40 (38%)*
FFP (units)	0 (0)	6 (4–8)*
Platelet (units)	0 (0)	1 (1–2)*
Cryoprecipitate (units)	0 (0)	2 (0–2)*
<b>Damage Control Procedure</b>		
Operating Room	-	61 (70%)
Interventional Radiology (IR)	-	6 (7%)
Combined OR/IR	-	4 (5%)

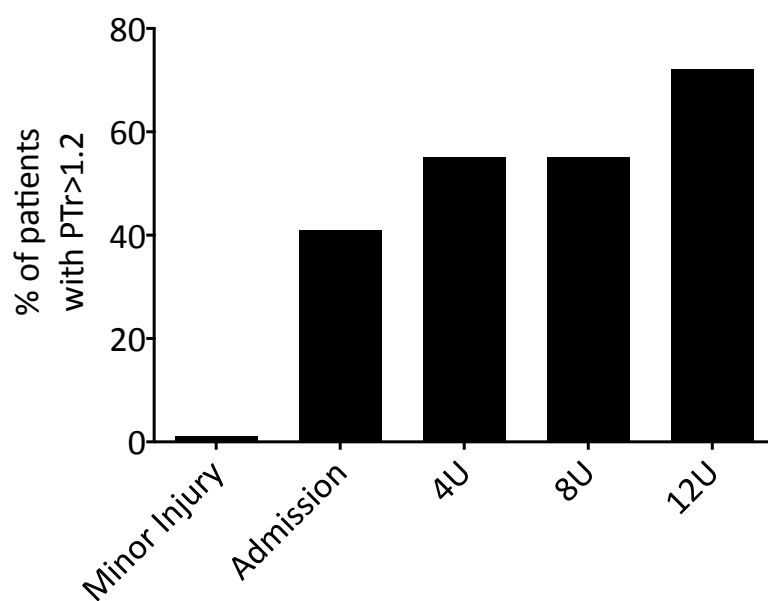
**Table 4.1. Patient demographics and clinical characteristics.** *Clinical characteristics of patients (n=106). Minor injury refers to those patients with an ISS≤4 from the ACIT II cohort. Values are given as median (interquartile range, IQR). \*p<0.05.*

Forty-three per cent (n=41) of bleeding patients were coagulopathic upon admission (*Table 4.1*). This proportion rose to 58% after four PRBC units and 81% after eight units (*Figure 4.1A*). Similarly, more patients developed a prolonged PTr during haemorrhage despite DCR and high plasma ratios (average six unit of FFP, two pools of CRYO and one pool of PLTs) (*Figure 4.1B*). The bleeding cohort had a significantly longer CT on admission ( $p<0.01$ ) as compared to the minor injury cohort (mean CT 88s vs 64s) as they continued to bleed. Patients who continued to bleed further had a gradual lengthening of their CT. The mean rate of clot formation also steadily deteriorated as patients bled and by 12 U, I found a 15% reduction in the alpha angle ( $p<0.01$ ). Clot strength (as measured by MCF EXTEM) was also reduced on admission in bleeding patients ( $p<0.001$ ). MCF continued to fall during DCR where, by the 12 U PRBCs, it had deteriorated by a further 19% ( $p<0.001$ , *Figure 4.1E*). The fibrinogen contribution to clot strength as measured by MCF FIBTEM also worsened in bleeding patients. Clot strength continued to worsen to 9 mm (*Figure 4.1F*) by the 12 U PRBCs, although this did not reach statistical significance. All functional clotting parameters deteriorated during haemorrhage, with the most significant changes seen in clot generation and clot firmness (*Figures 4.1C–F*). DCR protocols did not correct or improve TIC during the acute active haemorrhage.

**Figure 4.1. The evolution of TIC during haemorrhage.**



**Figure 4.1A.**



**Figure 4.1B.**

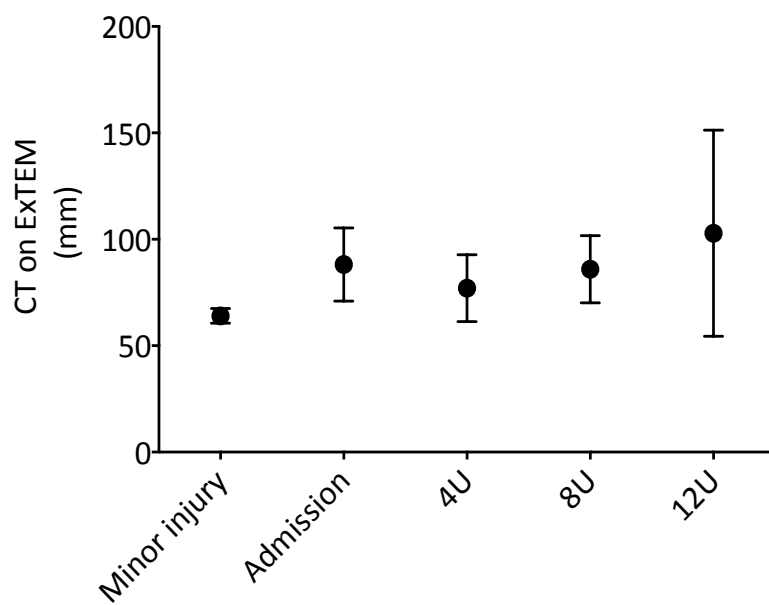


Figure 4.1C.

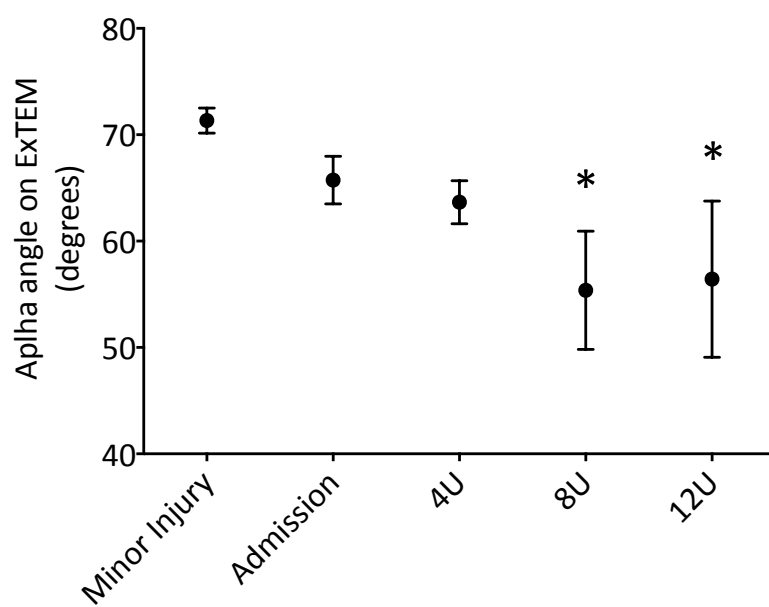


Figure 4.1D.



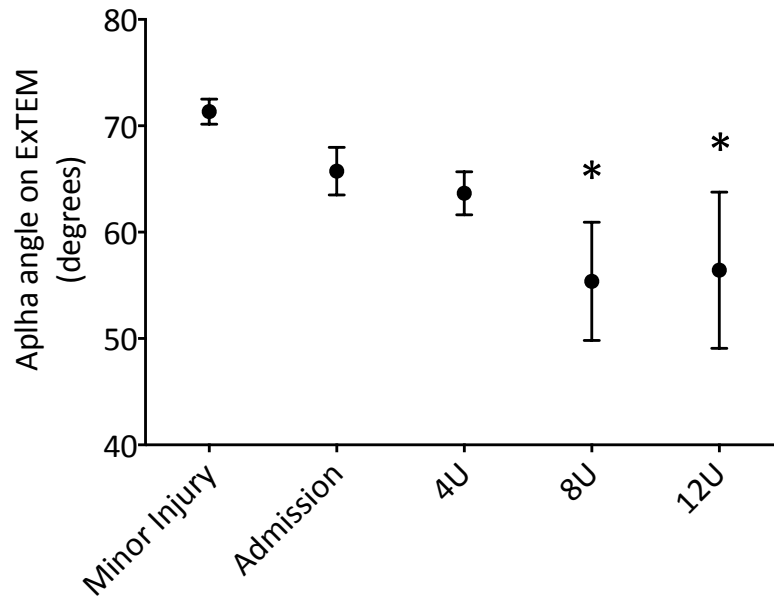


Figure 4.1E.

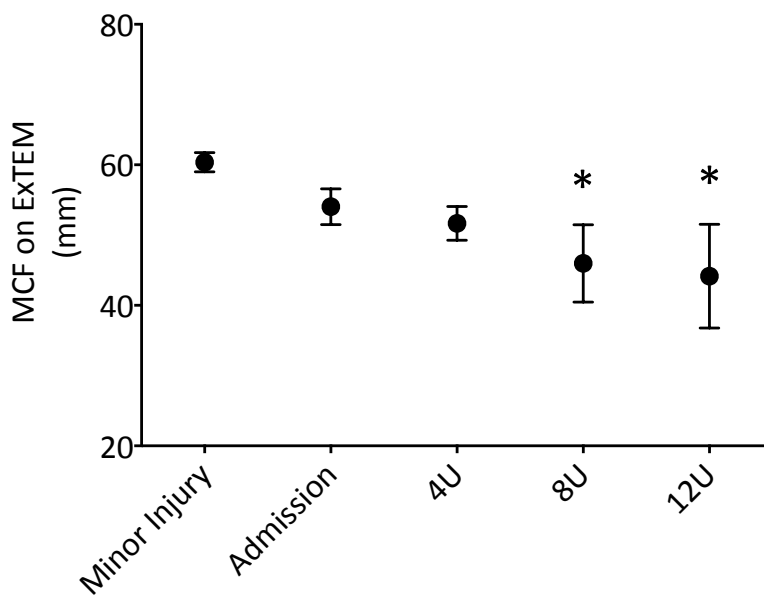
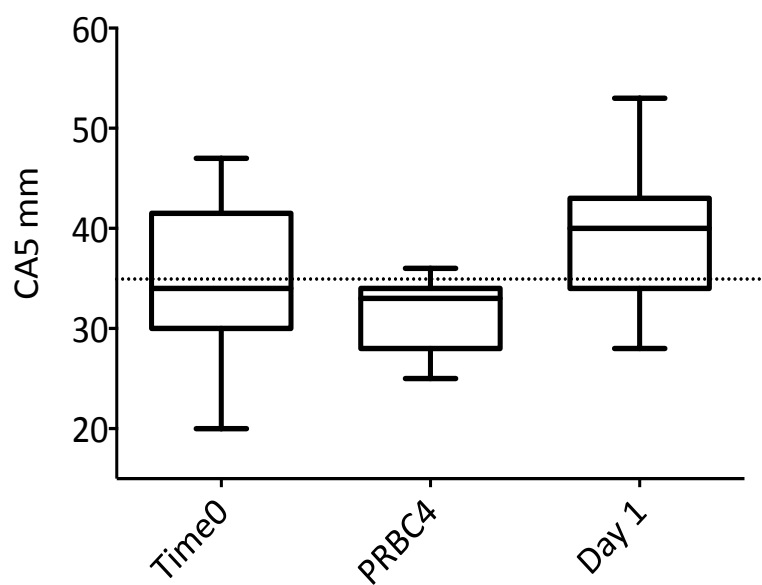


Figure 4.1F.

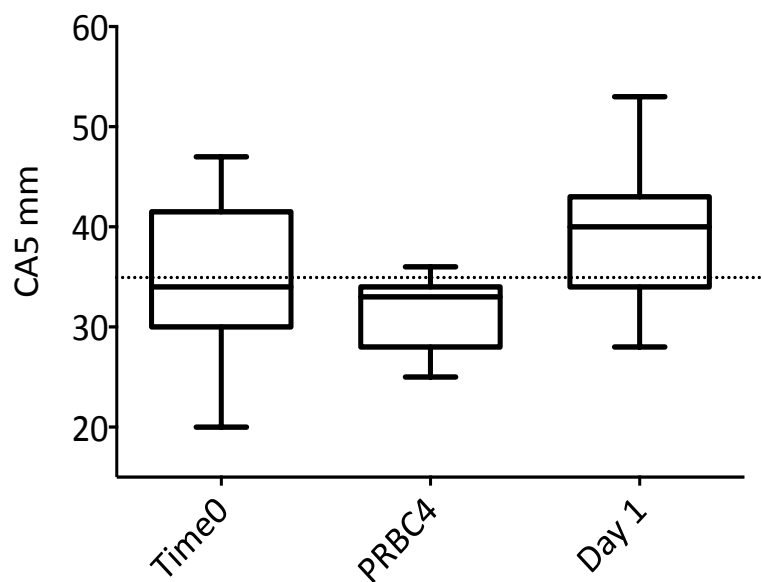
**Figure 4.1A–F. The evolution of TIC during haemorrhage.** Bars are mean with 95% CI. + Signifies comparison of admission vs minor injury. Minor injury refers to those patients with an  $ISS \leq 4$  from the ACIT II cohort. \* $p < 0.05$  Signifies a comparison to admission only. Minor injury values and upper/lower threshold for normal (---) ROTEM values for reference. A: Incidence of coagulopathy ( $CA5 \leq 35$ ) during trauma haemorrhage. B: Proportion of patients with  $PT(r) > 1.2$  during trauma haemorrhage. C: Mean CT on EXTEM. D: Mean alpha angle on EXTEM. E: Mean MCF on EXTEM. F: Mean MCF on FIBTEM.

The average clot amplitude values remained below the diagnostic threshold for coagulopathy ( $CA5 \leq 35\text{mm}$ ) throughout the bleeding episode (*Figures 4.2A–C*) with only partial correction evident on day one. During trauma haemorrhage, there was profound variability in individual responses to DCR (*Figure 4.2D*). Deterioration in maximum clot firmness was also observed during ongoing bleeding (*Figures 4.3A–C*). In the highest transfusion group, MCF deteriorated 15% during DCR and only improved on day one (PRBC 12+ U: Time 0, MCF 46 mm vs Day one MCF 59mm,  $p < 0.05$ ) (*Figure 4.3C*). Again, there was significant variability to DCR (*Figure 4.3D*). Prolonged CT ( $>94$  seconds) was observed in 33 patients (31%) despite DCR. This parameter also did not correct during ongoing bleeding (*Figures 4A–D*), with correction only observed on day one. A total of 65 patients (61%) had an abnormally flattened alpha angle  $<64^\circ$  (normal range  $63\text{--}81^\circ$ ) and, similar to the clot strength parameters, the angle deteriorated throughout the bleeding episode. Time 0: alpha  $59^\circ$ , PRBC 4–12 U: alpha  $54^\circ\text{--}55^\circ$ , day one: alpha  $70^\circ$  ( $p < 0.05$ ).

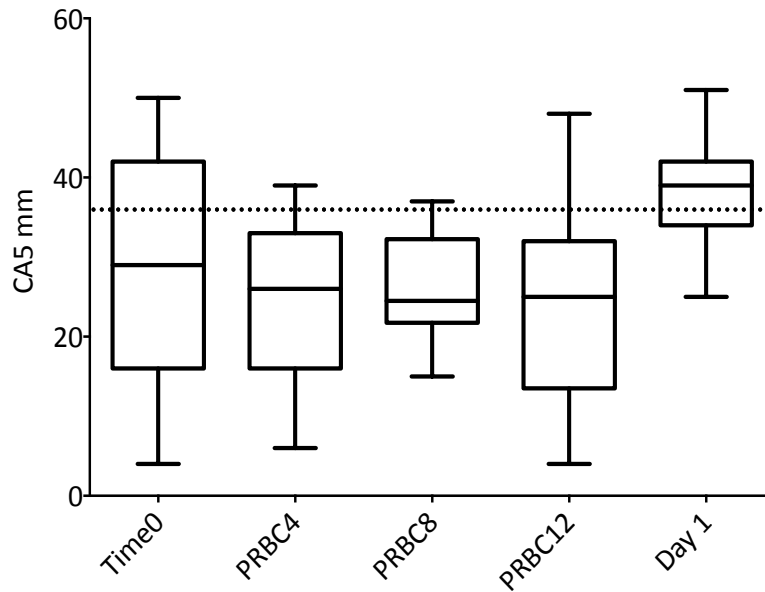
**Figure 4.2. Change in CA5 (mm) during bleeding episode in coagulopathic patients (CA<35 mm) stratified by transfusion requirements.**



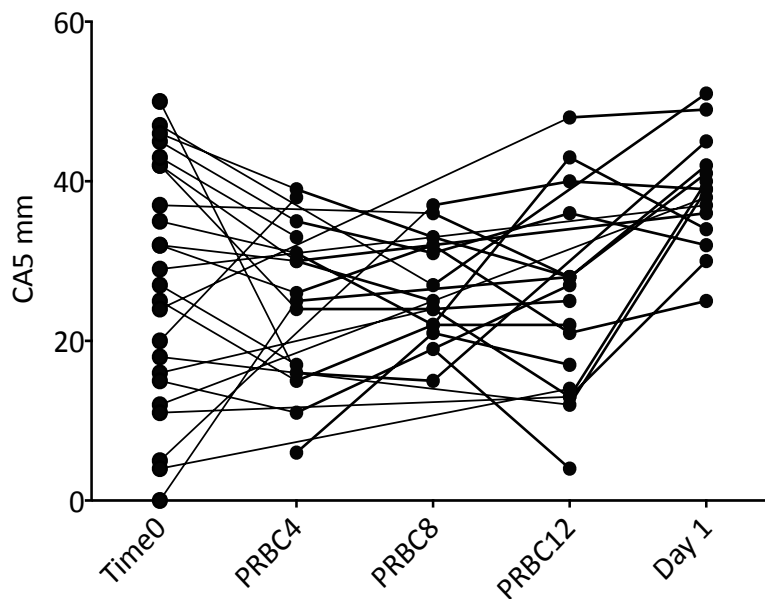
**Figure 4.2A.**



**Figure 4.2B.**



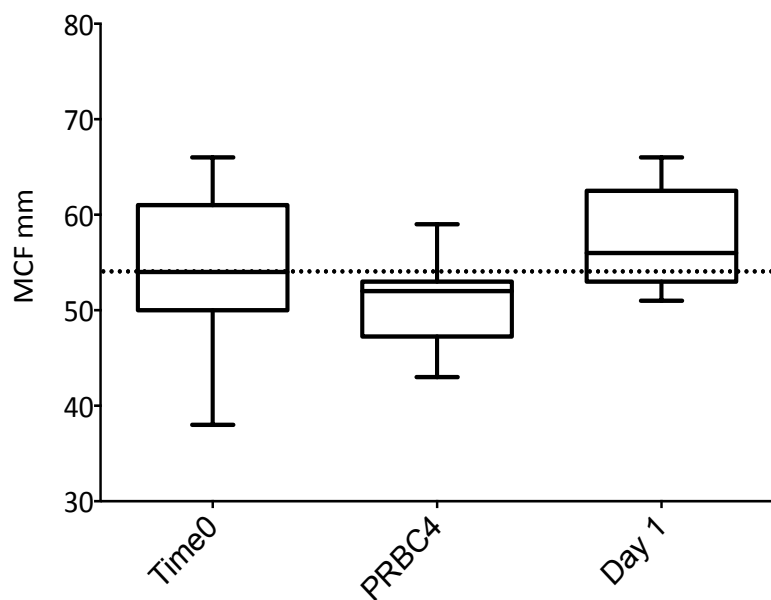
**Figure 4.2C.**



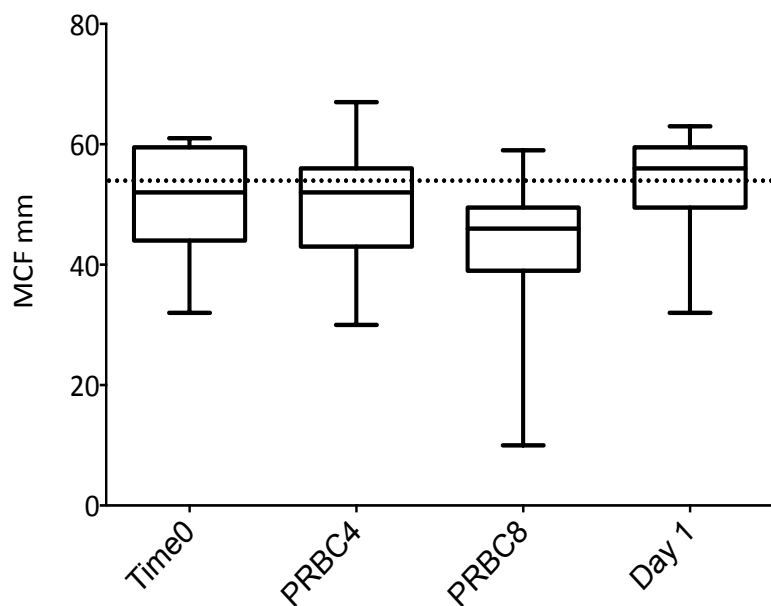
**Figure 4.2D.**

**Figure 4.2A–D. Change in CA5 (mm) during bleeding episode in coagulopathic patients ( $CA5 \leq 35$  mm) stratified by transfusion requirements.** The dotted line is the diagnostic threshold for ATC ( $CA5 \leq 35$  mm). A: Patients receiving 4 U to 7 U of PRBCs. Time 0: CA5, 35 mm vs day one: CA5, 39 mm ( $p < 0.05$ ). B: Patients receiving 8 U to 11 U of PRBCs. Time 0: CA5, 33 mm vs day one: CA5, 32 mm ( $p = 0.78$ ). C: Patients receiving 12 U or more PRBCs. Time 0: CA5, 27 mm vs day one: CA5, 39 mm ( $p < 0.05$ ). D: Individual responses in all patients receiving four or more units of PRBCs.

**Figure 4.3. Change in MCF (mm) during bleeding episode in coagulopathic patients (MCF < 54 mm) stratified by transfusion requirements.**



**Figure 4.3A**



**Figure 4.3B.**

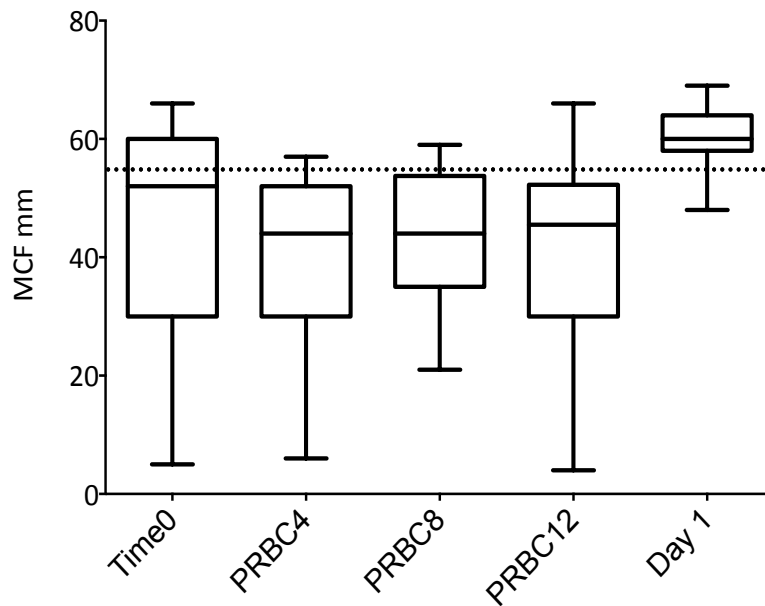


Figure 4.3C.

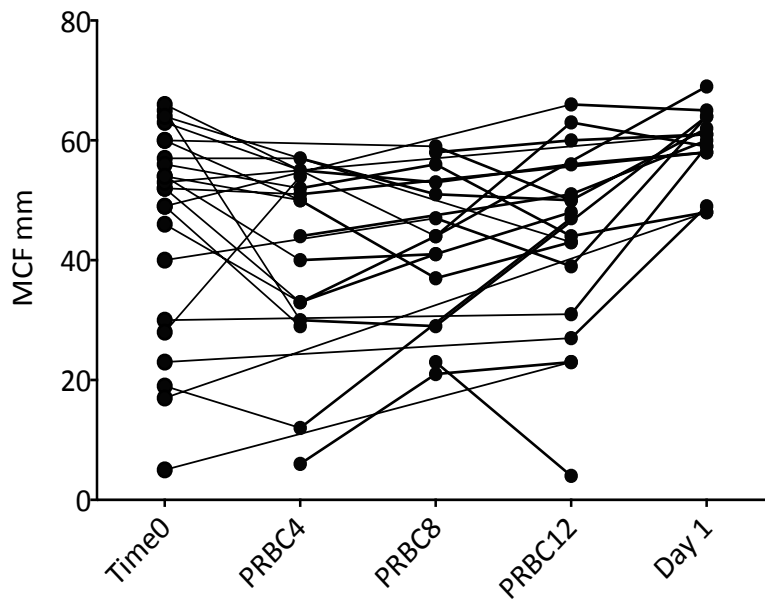
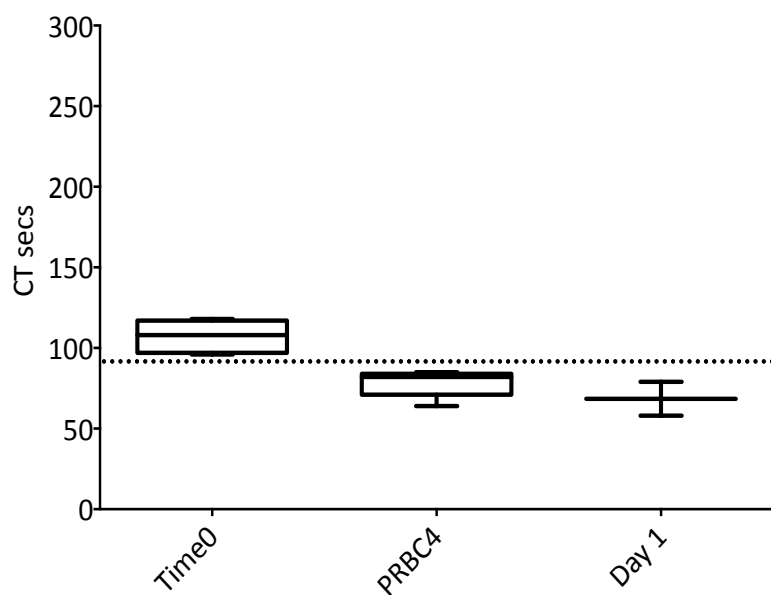


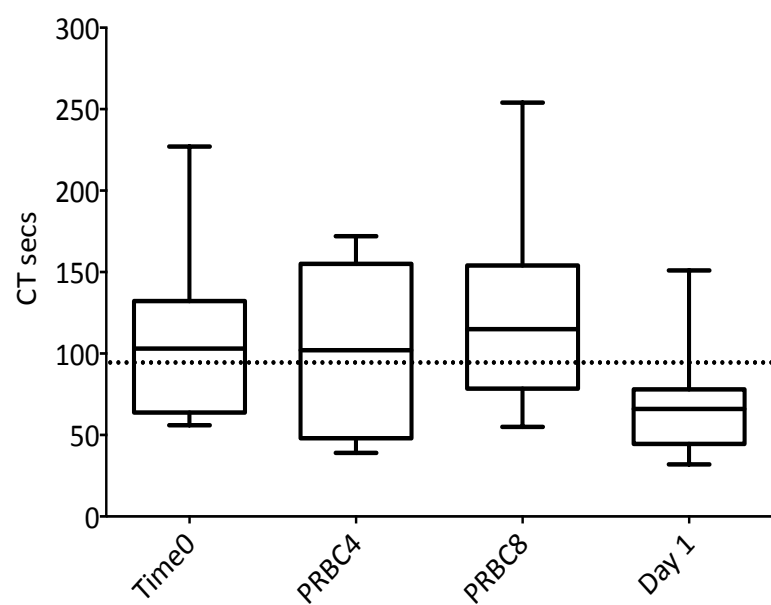
Figure 4.3D.

**Figure 4.3A–D. Change in MCF (mm) during bleeding episode in coagulopathic patients (MCF  $\leq$  54 mm) stratified by transfusion requirements.** The dotted line is the diagnostic threshold for ATC (MCF  $\leq$  54 mm). A: Patients receiving 4 U to 7 U of PRBCs. Time 0: MCF, 55 mm vs day one: MCF, 58 mm (insignificant). B: Patients receiving 8 U to 11 U of PRBCs. Time 0: MCF, 51 mm vs day one: MCF, 53 mm (insignificant). C: Patients receiving 12 U or more PRBCs. Time 0: MCF, 46 mm vs day one: MCF, 59 mm ( $p < 0.05$ ). D: Individual responses in all patients receiving four or more units of PRBCs.

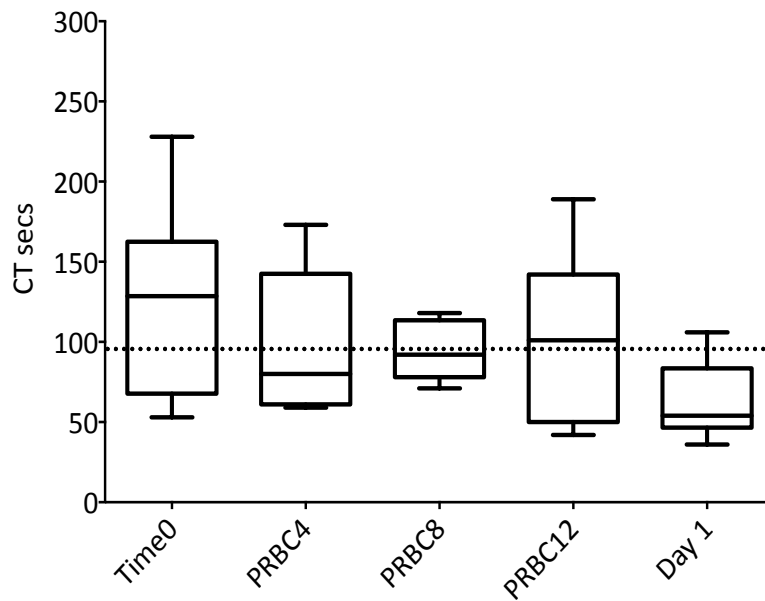
**Figure 4.4. Change in CT (secs) during bleeding episode in coagulopathic patients (CT > 94 secs) stratified by transfusion requirements.**



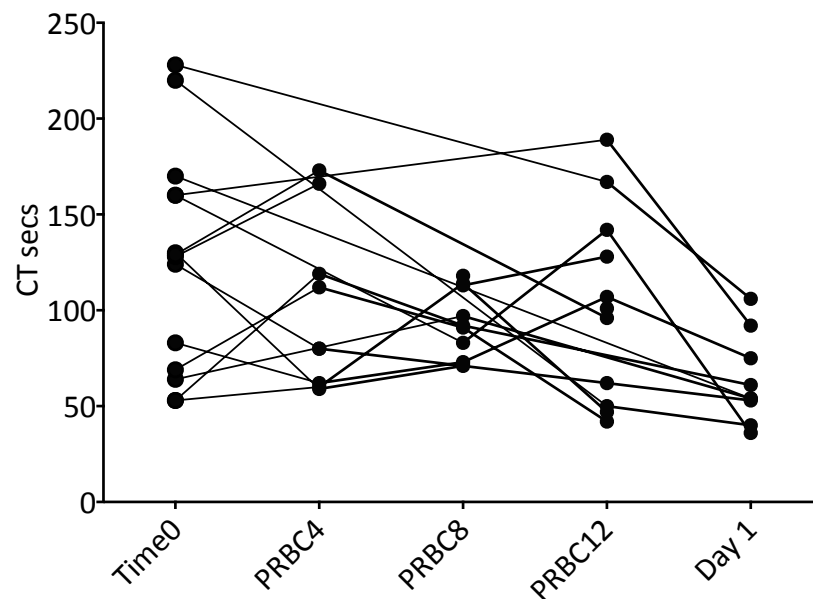
**Figure 4.4A.**



**Figure 4.4B.**



**Figure 4.4C.**



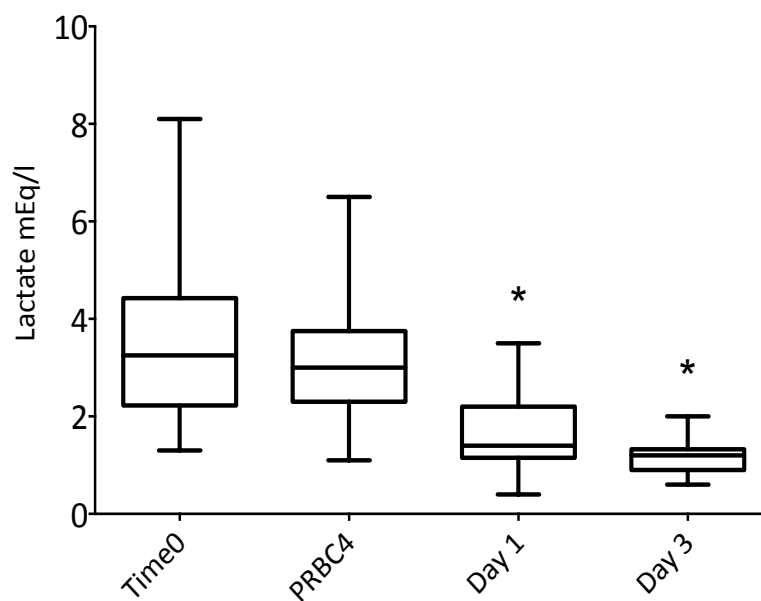
**Figure 4.4D.**

**Figure 4.4A–D. Change in CT (secs) during bleeding episode in coagulopathic patients (CT > 94 secs) stratified by transfusion requirements.** The dotted line is diagnostic threshold for ATC (CT 94 seconds). A, Patients receiving 4 U to 7 U of PRBC. Time 0: CT, 108 seconds versus Day 1: CT, 69 seconds ( $p < 0.05$ ). B, Patients receiving 8 U to 11 U of PRBC. Time 0: CT, 107 seconds versus Day 1: CT, 69 seconds ( $p = 0.07$ ). C, Patients receiving 12 U or more PRBCs. Time 0: CT, 126 seconds versus Day 1: CT, 63 seconds ( $p < 0.05$ ). D, Individual response in all patients receiving four or more PRBC units.

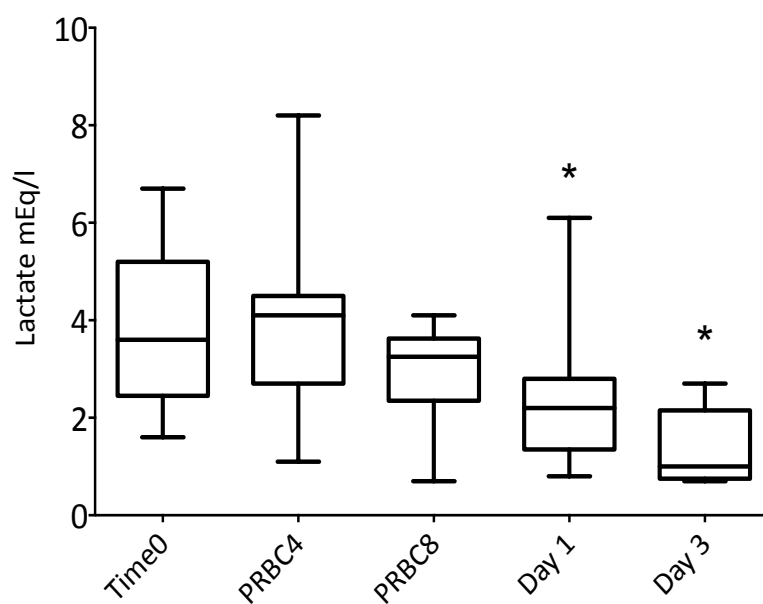


The average admission lactate was 6.2 mEq/l. Increased transfusion requirements were associated with higher baseline lactates (PRBCs four to seven U: Lactate 3.2 mEq/l vs PRBCs 12+ U: Lactate 9.2mEq/l,  $p<0.05$ ). Lactate levels remained elevated during the bleeding episode regardless of overall transfusion (*Figures 4.5A–C*). Patients who received up to seven units of PRBCs did not correct lactate levels until transfusion was complete (*Figure 4.5A*). Similarly, patients requiring eight to 11 units of PRBCs (*Figure 4.5B*) or 12+ units of PRBCs (*Figure 4.5C*) did not clear lactate during haemorrhage. Lactate levels were only normal (and significantly lower) on the day one sample (*Figure 4.5A–C*).

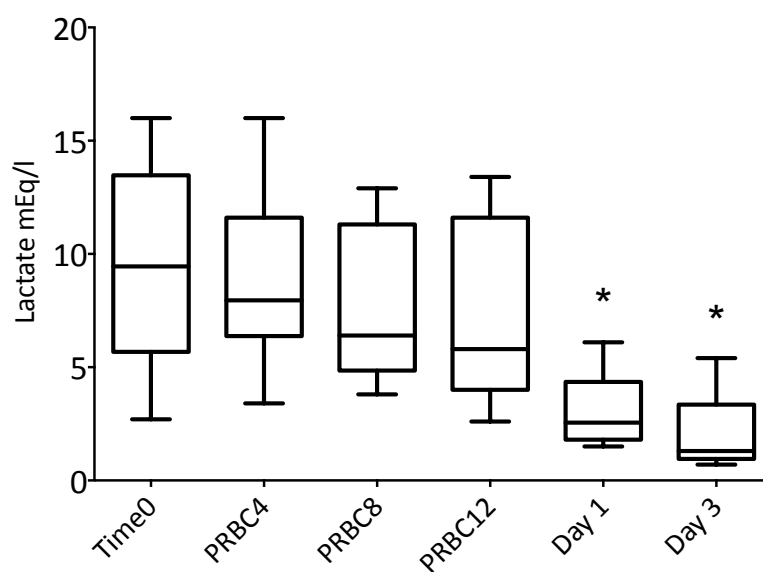
**Figure 4.5. Lactate clearance during haemorrhage.**



**Figure 4.5A.**



**Figure 4.5B**



**Figure 4.5C.**

**Figure 4.5A–C. Lactate clearance during haemorrhage.** *Lactate clearance during haemorrhage. A–C box and whisker plots—median, IQR and adjusted range. \*vs Time 0. A: Patients receiving PRBCs four to seven U. B: Patients receiving PRBCs eight to 11 U. C: Patients receiving PRBCs 12+ U.*

## 4.5 Discussion

This study describes the evolution of trauma haemorrhage in the severely injured, actively bleeding trauma patient. Large proportions of trauma patients are not only coagulopathic upon arrival to the ED, but more develop TIC during haemorrhage despite modern DCR practices. I have shown that DCR does not correct hypoperfusion or coagulopathy during the acute phase of haemorrhage. Furthermore, transfusion of FFP:PRBC ratios >1:2 throughout the bleeding episode failed to normalise any ROTEM parameters and lactic acidaemia did not clear until day one, after haemorrhage control had been achieved.

The paramount aim in treating exsanguinating trauma patients is immediate surgical control of the bleeding. Abdominal and extraperitoneal packing, closure of an open pelvic girdle and vascular embolisation are common measures to mechanically stop bleeding (103). Recently, there has been a paradigm shift in terms of treatment concepts that take into account contributors to TIC, including dilutional coagulopathy, hypovolaemic shock, hypothermia, acidaemia and hyperfibrinolysis. This has led to the concept of damage control surgery being extended to a more focused DCR, which implied permissive hypotension and fast provision of blood components. Severe acidosis, mostly a result of shock-related tissue hypoperfusion, impairs clotting factor activity, thrombin generation, platelet adhesion and aggregation, and enhances fibrinogen degradation (103, 181). Serum lactate measurements are a prognostic marker of tissue hypoperfusion and the severity of haemorrhagic shock in trauma (179, 182). Lactate clearance is correlated with survival and organ failure (183). Furthermore, correction of lactic acidaemia remains an important therapeutic goal of both DCR and surgery. Combining these two strategies in combat casualties has shown to correct metabolic derangements in a 'before and after' study of haemorrhage control (177). The results from this study are consistent with the current study's findings, but has also added to the body of knowledge by showing that restoration

of normal tissue perfusion and improvement in TIC does not occur until haemorrhage control is achieved.

Clot strength and clotting times deteriorated during ongoing bleeding, and only corrected when no further transfusion of PRBC units were required. These findings reinforce those from a comparative study of a lab-guided MHP vs empiric 1:1:1 (PRBC:FFP:PLT) protocol (94) (13). Fibrinogen, platelets and prothrombin time trends in the acute bleeding episode were identical in both transfusion strategies, despite a four-fold increase in patients receiving high FFP:PRBC ratio ( $>1:2$ ). DCR may offer several advantages over historical transfusion strategies, such as rapid access to empiric component therapy, but the findings from my study show it has limited efficacy in correcting TIC.

The benefits of DCR have been stated to be a reduction in crystalloid (CSL) use. Traditionally, in the presence of haemorrhagic shock, intravenous fluid infusion was the main treatment to improve tissue perfusion. However, aggressive replacement perpetuates the dilution coagulopathy and interstitial oedema, and impairs microcirculation, worsening oxygenation. Studies have shown that increased CSL use, in particular, is associated with dilutional coagulopathy and worse outcomes (35). Permissive hypotension is a strategy used to minimise or withhold fluid therapy or vasopressor support until the active bleeding has been controlled either by damage control surgery, or by radiologic or pharmacologic interventions. Untreated hypovolemic shock not only promotes tissue hypoperfusion but also hypoxia (184). Conversely, over utilisation of intravenous fluid resuscitation can increase blood pressure, thereby increasing hydrostatic forces on a newly developed thrombi. This could potentially displace the thrombi, resulting in a worsening of the haemorrhage. Permissive hypotension was practiced at all study sites, with limited volumes of CSL infused prior to baseline blood samples being taken in the ED. More severe changes in coagulopathy may have been seen had DCR protocols not been followed by centres in

this study. DCR may therefore somewhat protect from the deterioration of these parameters during bleeding but, in itself, is unable to correct TIC.

## **4.6 Limitations**

There are several limitations to this study. I was only able to sample up to the 12<sup>th</sup> PRBC unit transfused and therefore could not follow coagulation profile trends up to the point of haemorrhage control in some patients. The precise timing of haemorrhage control was difficult to ascertain and therefore, I used the completion of the PRBC transfusions as a surrogate measure for the control of bleeding. The average FFP:PRBC ratios were greater than 1:2 throughout, and I did not look specifically at the effect of differential ratios on coagulation or hypoperfusion. I also did not look at other component therapies (platelets and CRYO), although they will clearly influence correction of TIC. However, this is addressed, in part within the study reported in Chapter Five. In particular, fibrinogen is fundamental to haemostasis and falls to critical levels soon after the onset of major trauma haemorrhage (154). Early aggressive fibrinogen replacement may therefore prove efficacious in the correction of TIC and merits further investigation.

## **4.7 Conclusions**

The purpose of the study described in this chapter was to address the knowledge gap that exists regarding the evolution of TIC during trauma haemorrhage. This study shows that severely injured trauma patients are coagulopathic on arrival at the ED and that coagulopathy worsens during haemorrhage and DCR. Controlling haemorrhage and the cessation of transfusion appear necessary before TIC can be corrected and tissue perfusion restored. Following haemorrhage control and the completion of transfusions, ROTEM parameters do not immediately return to values seen in normal healthy controls. The question for the future design of goal-directed therapy-utilising devices such as ROTEM is

the identification of target values for coagulation parameters in trauma haemorrhage. Significant opportunities exist to tailor management and improve outcomes for bleeding trauma patients. A balanced and potentially novel strategy is required to achieve the correction of TIC while maintaining organ perfusion. Damage control resuscitative strategies as practiced by the institutions in this study may be protective but it is neither haemostatic nor resuscitative.

## CHAPTER FIVE

# **The effects of blood component therapy on coagulopathy during trauma haemorrhage**

## 5.1 Introduction

Chapter Four addressed the knowledge gap that exists in the incidence of TIC in an actively bleeding trauma patient. The ethos of DCR has been widely introduced among centres treating trauma patients with severe bleeding. This model concentrates on the early provision of coagulation products (in the form of plasma and platelet transfusions) as part of an MHP and addresses important confounders of the coagulation process, such as the lethal triad of coagulopathy, hypothermia, and acidosis. However, the efficacy of these blood components, as well as DCR overall, to correct TIC during acute bleeding is unknown.

The majority of studies supporting the use of high-dose blood components in trauma haemorrhage have retrospectively associated their use with survival, with the assumption that this is due to improved haemostasis during resuscitation (123, 177). However, observed survival benefits may be attributed to other factors, such as differential rates of bleeding and delayed provision of products, avoidance of crystalloid dilution or other unknown potential biases (46, 54, 124, 185). Our group has shown that physiological and haemostatic markers are not maintained during haemorrhage and DCR in the same study population (135, 154). A previous pilot study examined the ability of plasma to correct functional coagulation tests but did not find conclusive evidence that high-dose plasma offered a haemostatic benefit during bleeding (124). This study was small and therefore limited to the examination of one blood component only. To date, there have been no further prospective studies of the efficacy of type, dose or combinations of blood component therapies in correcting TIC during haemorrhage nor its effect on the coagulation profile during DCR.

Our research group has studied the bleeding phase following trauma and previously shown that fibrinogen, the primary substrate of coagulation, falls during haemorrhage. However,



despite DCR, fibrinogen did not correct with standard DCR (154). While high-dose plasma and platelets have both been associated with improved outcomes (94, 124), the preliminary findings showed that different ratios of FFP:PRBCs had minimal effect on the coagulation profile during trauma haemorrhage (124). It therefore remains unclear whether component therapy can improve coagulopathy in the acute phase.

## **5.2 Aims**

The hypothesis of this study is that blood component therapy improves trauma haemorrhage.

The overall objective is to describe the effect of blood component therapy on trauma haemorrhage.

The specific aims of my study are to describe the effect of individual blood components on TIC; and to examine the effect of increasing doses of FFP and fibrinogen for the correction of TIC.

## **5.3 Methods**

Trauma patients presenting directly to three Level 1 trauma centres between June 2010 and June 2013 were eligible for inclusion. Blood samples were collected as part of the ongoing prospective ACIT II investigation. All data and samples used in this study were consented for and the study was reviewed and approved by the respective National Research Ethics Committee of each country involved in the study.

### **5.3.1 Study design**

This study is undertaken with the same cohort of patients described in Chapter Four.

### **5.3.2 Patient selection**

This study is undertaken with the same cohort of patients described in Chapter Four. Patient selection is also as previously detailed in Chapter Four.

### **5.3.3 Sampling technique**

The sampling technique and analysis has been described in Chapter Four.

### **5.3.4 Definitions**

I defined coagulopathy by ROTEM as a five-min EXTEM Clot Amplitude (CA5)  $\leq 35$  mm (75). This has been shown to accurately identify ATC and predicts the need for MT (MT is defined as  $\geq 10$  units PRBCs in 24 h). The estimated expected fibrinogen doses transfused in each product are: PLT – 0.4g; FFP/Octaplas – 0.9g; and Cryoprecipitate – 2g for each pool/unit transfused (128).

### **5.3.5 Data collection and analysis**

Data were collected prospectively on patient demographics, time of injury, mechanism (blunt or penetrating), pre-hospital fluid administration, time of arrival at the ED, baseline vital signs, and total transfusion requirements in the first 12 h of admission. For each 4 U of PRBCs transfused, interval FFP:PRBC ratios were calculated up to the 12th PRBC unit. The change in coagulation parameters for each interval after four units of PRBCs was

calculated, e.g. time point A (baseline) to time point B (after the 4th, 8th and 12th Unit of PRBCs). Statistical analysis was performed using GraphPad PRISM v5 (GraphPad Software Inc, San Diego, CA, US) and Microsoft Excel 2007 (Microsoft, Inc., Redmond, WA, US). Normal quantile plots were used to test for normal distribution. Parametric data are expressed as mean  $\pm$  95% confidence intervals (CI). Non-parametric data are given as median (interquartile range, IQR). A  $p$  value of  $<0.05$  was chosen to represent statistical significance throughout.

## 5.4 Results

A total of 810 patients were included in the ACIT II study over study period with 49 exclusions: consent declined or not possible (21 patients) and retrospective exclusion criteria (28 patients). A total of 106 study patients were transfused  $\geq 4$  U PRBCs during the study period with 27 receiving 8–11 U PRBCs and 31 who received  $\geq 12$  U PRBCs. The average FFP:PRBC ratio between intervals was PRBCs 0–4 U: 0.5; PRBC 5–8 U: 0.9, PRBCs 9–12 U: 0.7 and at h 12: 0.7. Median crystalloid use was 0 ml for all intervals: PRBCs 0–4 U (IQR: 0–100 ml), PRBCs 5–8 U (0–500 ml), PRBCs 9–12 U (0–1,000 ml). Overall, mortality was 35%. Clinical characteristics, admission physiology, and laboratory parameters are detailed in *Table 5.1*. After the first interval, there were no differences in the ratio of any blood product transfused between the 4–8 and 8–12 unit intervals (*Table 5.2*).

In total there were 181 4-U PRBCs intervals available for analysis in this study (*Table 5.2*). Very few blood components other than PRBCs were delivered in the first 4-U window, as emergency blood is delivered from stocks held in EDs. On average, patients received 2 U of FFP with the first 4 U of PRBCs. Patients received significantly more of all components in subsequent 4-U intervals—on average, 3.4 U of FFP, 0.5 pools of platelets and 0.8 pools of CRYO, equivalent to 4.4g of fibrinogen (*Table 5.2*).

<i><b>Patients</b></i>	<i><b>n=106</b></i>
<b>Age</b>	44 (30–60)
<b>Male</b>	75 (76%)
<i><b>Injury</b></i>	
<b>ISS</b>	34 (25–41)
<b>ISS&gt;15</b>	99 (94%)
<b>Blunt injury</b>	92 (87%)
<i><b>Admission parameters</b></i>	
<b>SBP&lt;90 mmHg</b>	41 (46%)
<b>Base deficit&gt;6 mEq/L</b>	68 (67%)
<b>Lactate&gt;5 mEq/L</b>	44 (42%)
<b>PT (secs)</b>	12.9 (11.7–14.6)
<b>PTr&gt;1.2</b>	40 (40%)
<b>CA5≤35 (mm)</b>	41 (43%)
<i><b>Fluid requirements (first 12 h)</b></i>	
<b>Pre-hospital Crystalloid (ml)</b>	500 (100–2,000)
<b>Crystalloid (ml)</b>	2500 (1,300–4,000)
<b>Colloid (ml)</b>	875 (0–1688)
<b>PRBC units</b>	8 (5–13)
<b>PRBC≥10 U</b>	40 (38%)
<b>FFP units</b>	6 (4–8)
<b>Platelet units</b>	1 (1–2)
<b>Cryoprecipitate units</b>	2 (0–2)

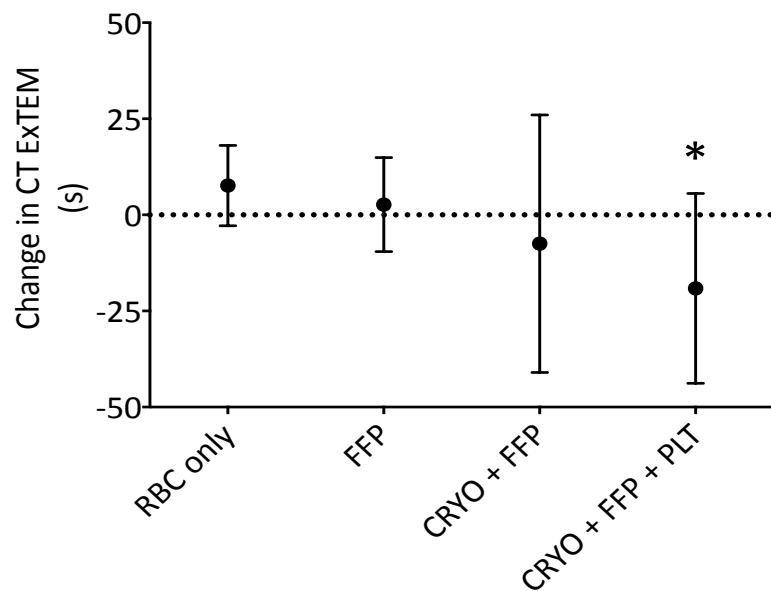
**Table 5.1. Clinical characteristics of patients.** *Values are given as n (%) or median (interquartile range).*

Blood component	0–4 unit interval	5–8 unit interval	9–12 unit interval
Number	106	49	26
Average FFP units	2.0	3.2*	3.7*
Average FFP:PRBCs	0.5	0.9*	0.7*
Average PLT units	0.2	0.4*	0.8*
Average PLT:PRBCs	0.0	0.1*	0.2*
Average CRYO units	0.2	0.6*	1.2*
Average CRYO:PRBCs	0.0	0.2*	0.3*
Total Fg transfused (g)	2.1	4.3*	4.7*

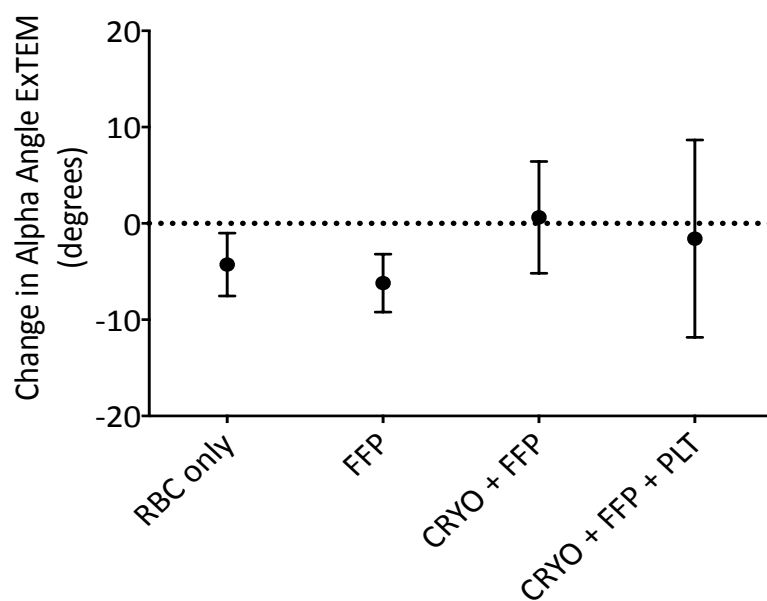
**Table 5.2. Blood components administered during DCR.** *Transfusion requirements for each interval of 4 U of PRBCs. There was no significant difference between the 5–8 U window and the 9–12 U window for any product transfused. \* $p < 0.05$  when compared to the 0–4 U interval + $p < 0.05$  when compared to the 4–8 U interval.*

Blood component therapy was compared to the transfusion of PRBCs alone (*Figures 5.1A–D*). There was little discernible effect on the clotting function regardless of the type of blood component administered. The average dose of FFP administered per interval (3.2 U) had no significant effect on clotting time, clot generation nor maximum clot firmness (*Figures 5.1A–2D*). When adding CRYO (an average of 1.6 pools per interval) and platelets (an average of 1 pool per interval), this showed some level of protection for the clotting function across the interval, but only an improvement in CT time was significant (+7.6 s vs. -19.2 s  $p < 0.05$ ). There were very few intervals where platelets or CRYO was transfused in isolation; thus I could not identify the individual effect of these products. Component therapy in average doses appears to have little effect on functional clotting profile during acute haemorrhage.

**Figure 5.1. Effect of blood component therapy on coagulopathy when administered during DCR.**



**Figure 5.1A.**



**Figure 5.1B.**

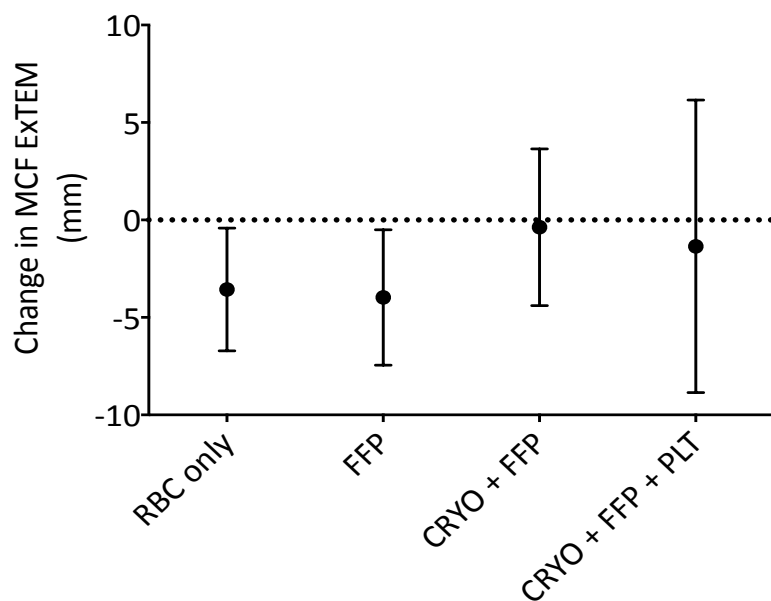


Figure 5.1C.

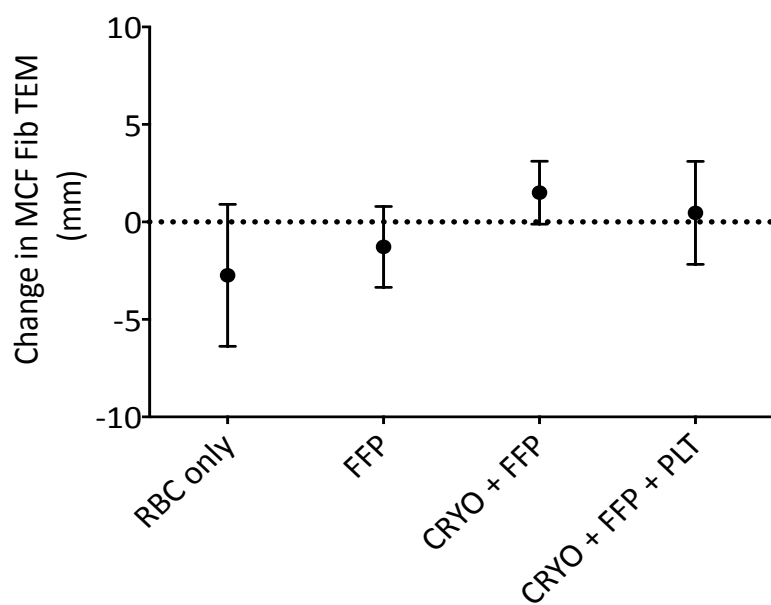


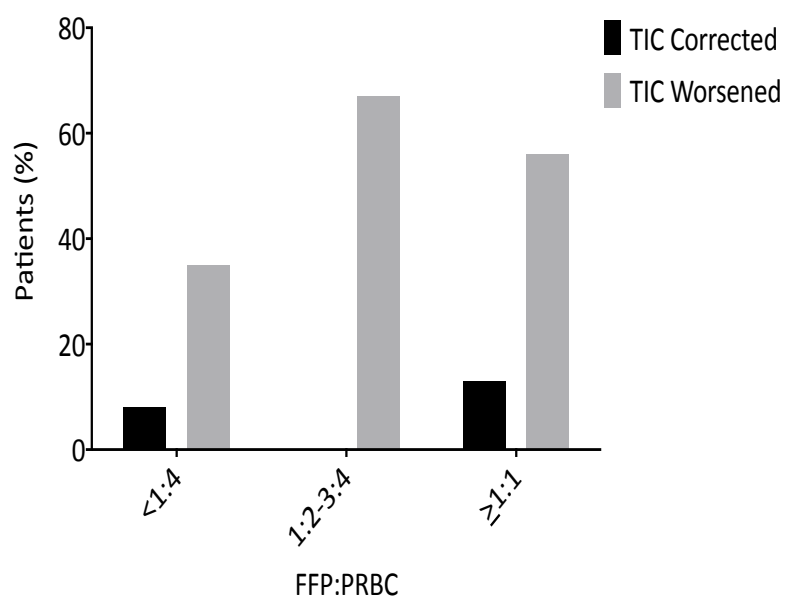
Figure 5.1D.

**Figure 5.1A–D. Effect of blood component therapy on coagulopathy when administered during DCR.** Bars are mean interval change in ROTEM parameters with 95% CI. A: Change in CT on EXTEM. B: Change in alpha angle on EXTEM. C: Change in MCF on EXTEM. D: Change in MCF on FIBTEM.

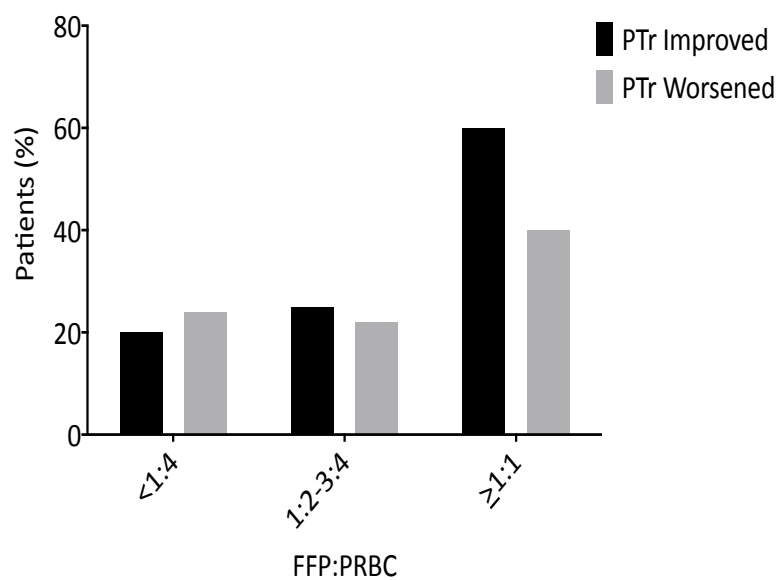
There was sufficient variation in FFP administered between intervals to allow an analysis of the effect of FFP dose on the coagulation function. Overall, less than 10% of coagulopathic patients had their coagulopathy corrected at the end of the 4-U interval, regardless of the amount of FFP administered (*Figure 5.2A*). Increasing doses of FFP also did not prevent patients from becoming coagulopathic, with 56% of intervals that started with CA5>35 mm becoming coagulopathic with 1:1 FFP:PRBC administration, compared to 35% with 1:4 ratios (*Figure 5.2A*). Increasing doses of FFP had a slightly better effect on the PTr, with 60% of intervals that started with a PTr>1.2 normalising with a 1:1 ratio, compared to less than 20% with a 1:4 ratio (*Figure 5.2B*). However, on average, all parameters of the coagulation function deteriorated in all intervals regardless of the dose of FFP administered (*Figures 5.2C–F*). There were more consistent findings for the effect of the total dose of fibrinogen administered within the blood components. Fibrinogen doses of six g or more achieved correction of CA5 and PTr in 36% and 29% of patients respectively (*Figures 5.3A–B*). There were statistically significant improvements in functional clotting parameters when six g or more of fibrinogen was administered within a 4-U PRBC interval (*Figures 5.3C–F*). However, 57% of intervals that started with normal functional clotting developed coagulopathy despite this dose of fibrinogen (*Figure 5.3A*). Although there was a wide variability in the fibrinogen response, higher dose replacement offered the most consistent improvements in functional clot measures during haemorrhage.



**Figure 5.2. The effect of increasing doses of FFP on TIC.**



**Figure 5.2A.**



**Figure 5.2B.**

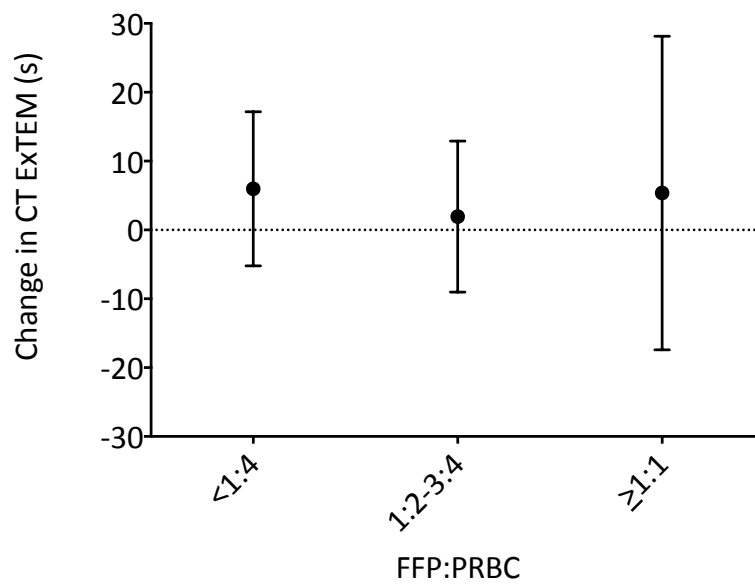


Figure 5.2C.

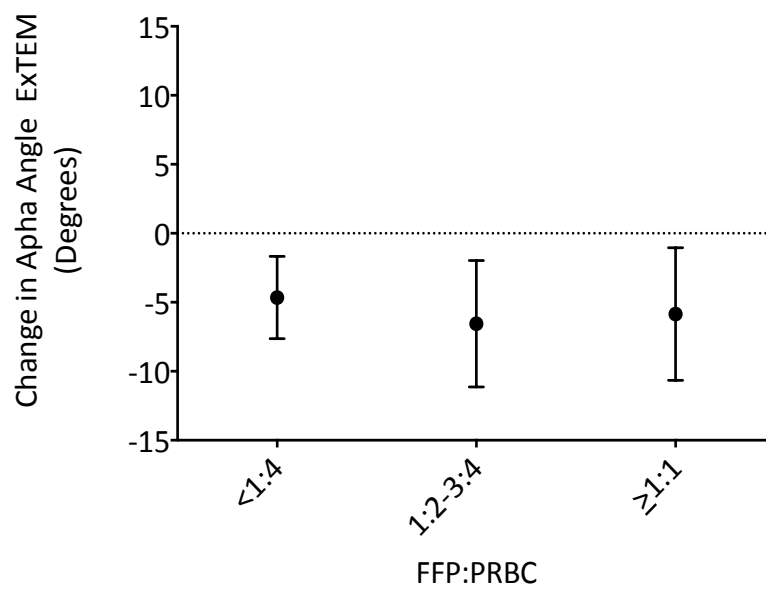
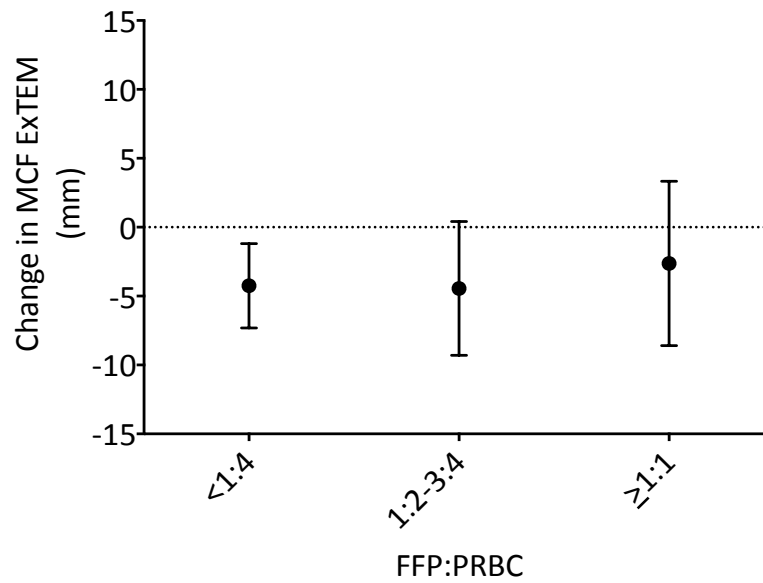
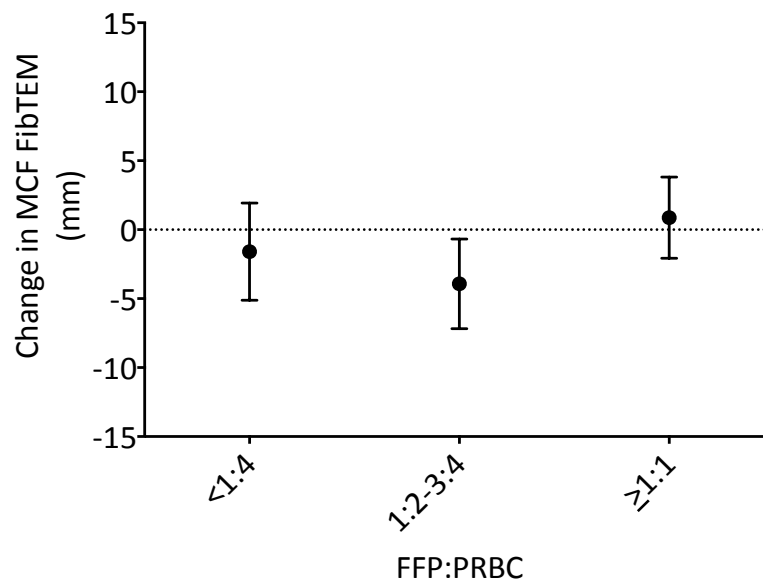


Figure 5.2D.



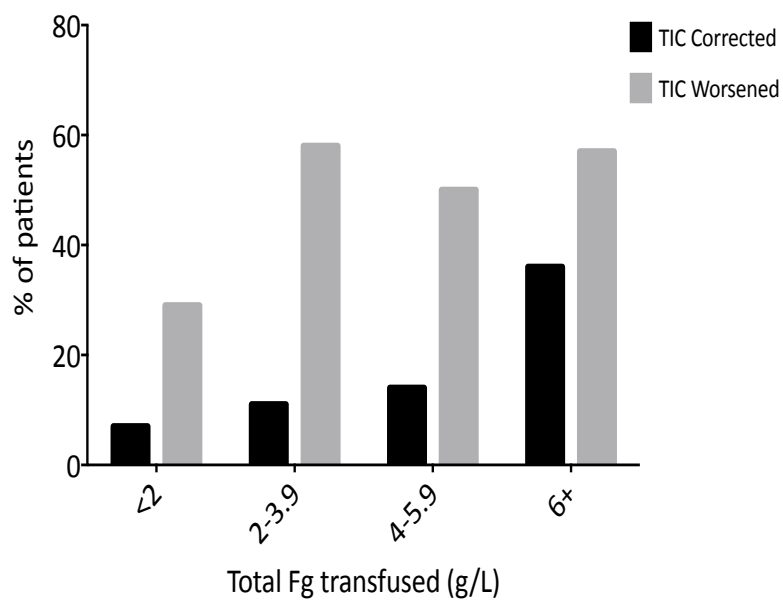
**Figure 5.2E.**



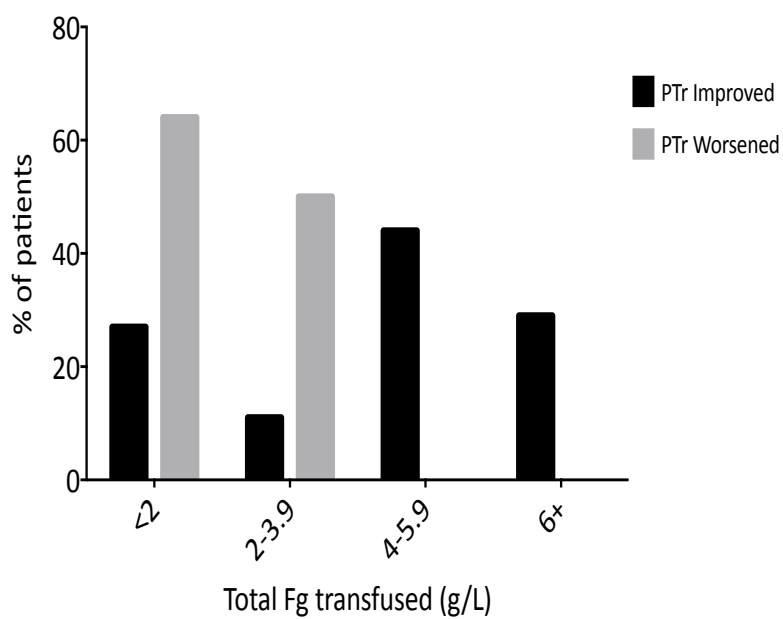
**Figure 5.2F.**

**Figure 5.2A–F. The effect of increasing doses of FFP on TIC.** Bars are mean change in ROTEM parameters with 95% CI. A: The effect of increasing FFP:PRBCs on the proportion of trauma patients with coagulopathy corrected ( $CA5 \geq 35$ ) and coagulopathy worsened ( $CA5 \leq 35$ ). B: The effect increasing FFP:PRBCs compared to the proportion of patients with corrected PT(r) and worsening PT(r). C: Average CT on EXTEM. D: Average alpha angle on EXTEM. E: Average MCF on EXTEM. F: Average MCF on FIBTEM. There was no difference between the FFP:PRBC ratio groups in any measurement of the coagulation response.

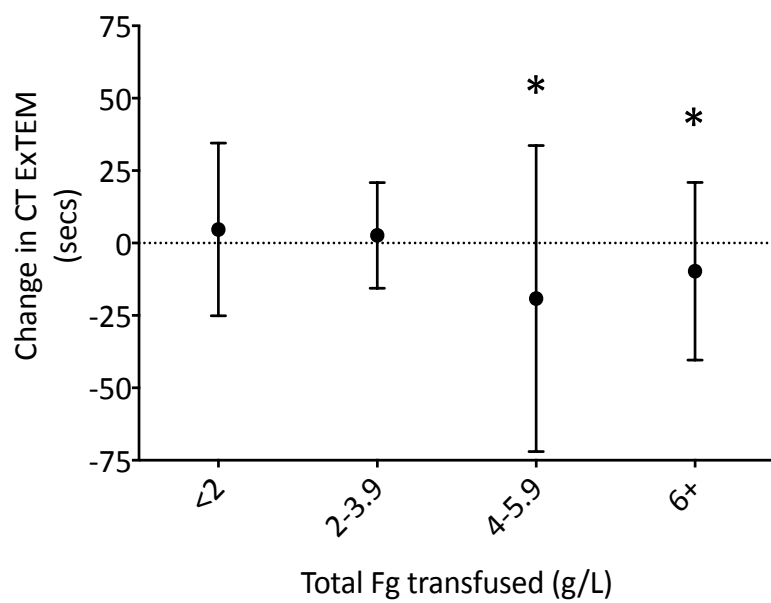
**Figure 5.3. The effect of increasing interval fibrinogen doses on TIC.**



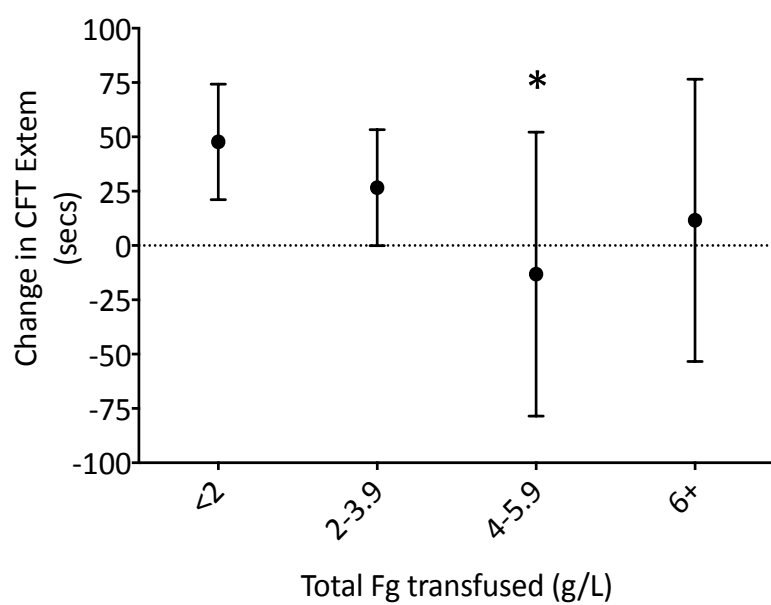
**Figure 5.3A.**



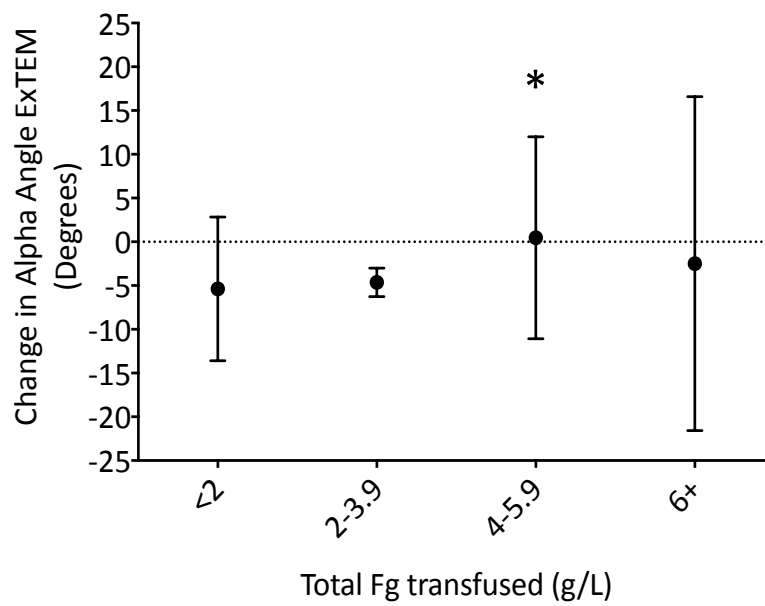
**Figure 5.3B.**



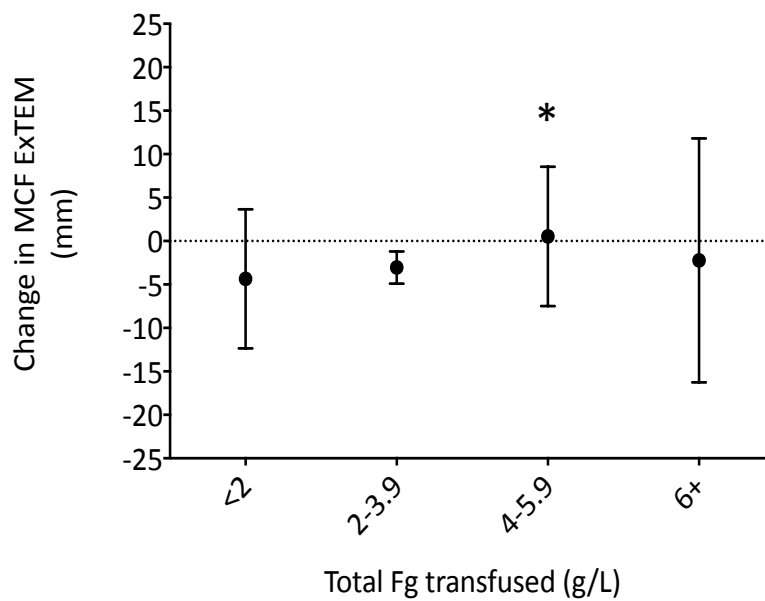
**Figure 5.3C.**



**Figure 5.3D.**



**Figure 5.3E.**



**Figure 5.3F.**

**Figure 5.3A–F. The effect of increasing interval fibrinogen doses on TIC.** Bars are mean change in ROTEM parameters with 95% CI. A: Change in CT on EXTEM. B: Change in alpha angle on EXTEM. C: Change in MCF on EXTEM. D: Change in MCF on FIBTEM.

## 5.5 Discussion

In this prospective study of severely injured, bleeding patients, I have shown that high-dose blood component therapy is generally able to maintain coagulation parameters but cannot correct TIC. It is only at the highest dose of combinations of products that a consistent, although small, effect was observed within this population.

Despite the widespread use of blood components to treat TIC, their effects on the coagulation profile when administered during DCR remains unknown. There are many clinical studies that emphasise the importance of the identification and treatment of TIC early in the clinical course of the bleeding trauma patient (186). However, some studies suggest that, even in patients where clinically apparent coagulopathy is addressed with blind early administration of blood products, namely FFP, the amount transfused often falls significantly below what is required to address the complex coagulopathy related to dilution, consumption, and fibrinolysis (46, 187). Previous studies have investigated the effect of varying FFP:PRBC on outcomes, but there has been conflicting evidence regarding the use of high ratios. High-dose plasma and platelets have both been associated with improved outcomes (8), but our previous pilot study found that increasing ratios of FFP:PRBCs had a variable effect on the coagulation profile during haemorrhage (124). Through this study, I have shown that there was no consistent correction of any measure of clot function when FFP was delivered during the acute phase of ongoing bleeding. Furthermore, I could not identify a major haemostatic benefit to FFP:PRBC ratios above 1:2.

Normal haemostasis is critically dependent on fibrinogen as a substrate for clot formation. The fibrinogen level is decreased in the severely injured, bleeding patient on admission and is associated with poor outcomes (130). Our group has previously shown that fibrinogen

depletion occurs in TIC and progresses during trauma haemorrhage (130, 161). Fibrinogen levels do not normalise during DCR despite the provision of high ratios of both plasma and platelets. There was a small but inconsistent response to high doses of fibrinogen in this current study. This may be due to the volume of blood components that have to be administered to achieve such high doses in a standard DCR protocol. For example, four units of FFP, one pool of platelets and one pool of CRYO would provide six g of fibrinogen, but diluted into a volume of approximately three l (when given with four units of PRBCs). It is possible that a more concentrated delivery may be more effective, and clinical trials of early high-dose CRYO therapy and fibrinogen concentrate are underway.

## **5.6 Limitations**

There are several limitations to this study. Firstly, this was an observational study and thus, results can only show the effect of blood components as administered by the MHPs in our institutions. Patients received different blood components across different PRBC intervals, and were more likely to receive 1:1 FFP:PRBC ratio between PRBC units 8 and 12. These interval cohorts may be influenced by our transfusion protocols and practices, rate of blood loss, or early haemorrhage control. The effect of immediate balanced transfusion therapy with plasma, platelets and RBCs was not evaluated in the present study. Randomised control trials of plasma and other blood component therapies in TIC are highly warranted (161). While I have excluded survival bias in this study, there may still be differences in these parameters that may have influenced the observed efficacy of the component therapies. Precise timing of haemorrhage control is difficult to ascertain, and therefore, I used the completion of PRBC transfusions as a surrogate measure for the control of bleeding. I did not examine the fibrinolytic component of TIC in this study, although from 2010 and the publication of the CRASH-2 trial, MHPs at all sites specified early administration of tranexamic acid. Under-treated or persistent hyperfibrinolysis may contribute to poor clot function and is therefore part of a continued investigation within



INTRN. Finally, 28 patients were logistically impossible to sample as the rate of transfusion meant I was unable to sample every 4 U of PRBCs. As I was not studying outcomes, this will not have introduced bias to our results, but this is clearly an important group of patients who I was not able to study.

## **5.7 Conclusion**

The purpose of this study is to address the knowledge gap that exists regarding the efficacy of blood component therapy on TIC during active bleeding. I have shown that severely injured trauma patients were coagulopathic on arrival at the ED and early replacement of increasing doses of plasma and average doses of other blood component therapies had little effect on the deranged ROTEM coagulation parameters during the acute phase of care. I have shown that aggressive high-volume replacement of plasma has little effect on deranged ROTEM coagulation parameters. A better understanding of the pathogenesis of TIC is required to delineate the limitations of current DCR strategies in the treatment of major trauma haemorrhage and in the identification of novel therapeutic targets. There is an important opportunity to improve the efficacy of DCR by improved the management of TIC during haemorrhage.

## CHAPTER SIX

# **The effects of damage control resuscitation on coagulation factor concentration during trauma haemorrhage**

## 6.1 Introduction

DCR is the administration of early and aggressive clotting factor transfusions in the form of high-dose FFP and CRYO in combination with PRBCs, while simultaneously avoiding large volumes of crystalloid transfusion (117, 170, 185, 188). The effect of DCR on coagulation-factor activity levels in the acute bleeding phase is an area of continued uncertainty but is of obvious importance. In the last two decades, there have been numerous studies, albeit all retrospective, that have shown that the early transfusion of plasma, containing coagulation factors, improves survival (38, 40, 41, 123, 189, 190). The first of these major investigations that examined DCR was carried out by Borgman et al. at a US combat hospital in Iraq. This retrospective study examined 252 patients who had been given a MT, and found significant differences in mortality between patients who were given low, medium or high plasma to red blood cell ratios. The higher the plasma ratio in the blood, the lower the risk of death. This was achieved primarily by reducing early mortality (less than four h from admission) from haemorrhage (123). This study was limited by the lack of standard timing for measuring variables, and the lack of a MT protocol that would be consistently applied to patients. Other studies in military trauma have also shown a mortality benefit from high plasma to red blood cell ratios (41, 47, 123, 191). Holcomb described similar results in 466 MT civilians (40). The ideal ratio of PRBC to FFP is controversial, with some studies proposing that such high ratios do not have any additional benefits over medium ratios (124). A recent large, prospective cohort study documenting the timings of transfusions during active resuscitation and patient outcomes concluded that, in the first six hours, patients with ratios less than 1:2 were three to four times more likely to die than patients with ratios of 1:1 or higher (130). Nonetheless, it is clear from the literature that there exists a knowledge gap, as relatively little is known of the effect of blood component therapy on coagulation factor concentration during the acute phase of bleeding (54, 119, 123, 134).

Recent evidence has shown that there is an early clotting factor deficiency on admission that is associated with hypoperfusion (190, 192). Studies have reported that up to 20% of patients had at least one clotting factor deficiency on arrival, defined as less than 30% activity or concentration (192). However, the designation of critical coagulation factor activity levels associated with clinically relevant abnormalities is based on the study of patients with hereditary single-factor deficiencies. The effects of multiple subcritical deficiencies, as seen in trauma patients, are not well understood. Further evidence regarding clotting factor deficiency contributing to ATC has come from the PROMMT study (130). In addition to DCR, where many proteins are replaced concurrently, isolated factors such as recombinant factor VII (rFVII) have been proposed as treatments for injured patients with haemorrhagic shock (193). However, transfusing clotting factors in the form of FFP or CRYO implicitly refutes the hypothesis that clotting factor deficiency is implicated in early TIC (192). It is important to understand what effect the type, dose and combinations of blood component therapies have on coagulation factor level in the severely injured, bleeding trauma patient.

## **6.2 Aims**

The hypothesis of this study proposes that blood component therapy maintains coagulation factor concentrations during trauma haemorrhage.

The overall objective is to characterise the effect of DCR and blood component therapy on coagulation factor concentrations during trauma.

The specific aims are to characterise the evolution of coagulation factor concentrations during the acute phase of trauma haemorrhage. The second aim is to describe the effect of TIC on coagulation factor concentrations. The third aim is to examine the effect of

individual blood components on coagulation factor concentrations. Finally, the fourth aim is to determine the effect of increasing doses of FFP on coagulation factor concentrations.

## **6.3 Methods**

### **6.3.1 Study design**

This was a multicentre, prospective, cohort study of trauma patients presenting directly to level 1 trauma centres between June 2010 and June 2013. The study was reviewed and approved by the UK National Research Ethics Committee or equivalent (as described in Chapter Two). I analysed baseline laboratory coagulation parameters on arrival for comparison with coagulation factors, ROTEM and BD.

### **6.3.2 Patient selection**

All adult trauma patients ( $\geq 16$  years) who met the local criteria for full trauma team activation were eligible for enrolment and recruited into the study when research personnel were present (08:00 to 22:00 daily). Exclusion criteria included arrival at the ED more than two h after injury; the administration of more than 2,000 ml of intravenous fluid prior to arrival at the ED; transfer from another hospital; and burns covering more than 5% of the total body surface area. Patients were retrospectively excluded if they declined to give consent for the study to use research samples collected, were found to be taking anticoagulant medications, and had moderate or severe liver disease or a known bleeding diathesis. Clinicians actively involved in patient treatment were blinded to ROTEM results. Further details of methodology are given in Chapter Two.

### **6.3.3 Sampling technique**

A 30 ml research sample of blood was drawn from either the femoral vein or antecubital fossa along with the standard trauma laboratory tests within 20 minutes of the patient's arrival in the ED. Samples for PT, fibrinogen and coagulation factor assay were collected into 4.5 ml glass vacutainers (0.109 M buffered sodium citrate, 3.2%: Becton Dickinson, Plymouth, UK). PT and fibrinogen were processed by the central hospital along with a standard FBC. PTr was calculated as observed: PT divided by mean control PT values in order to enable direct comparison. Blood for ROTEM analysis was drawn into a 2.7 ml citrated vacutainer (0.109 M buffered sodium citrate, 3.2%: Becton Dickinson, Plymouth, UK) and 97 were processed in the trauma research laboratory. Arterial blood analysis for BD was performed simultaneously with the research sample collection. For those patients with acute active bleeding that required immediate transfusion as part of DCR, further blood samples were drawn after the 4th, 8th and 12th unit of PRBC were transfused. Only patients that were transfused with more than 4 U PRBCs within 12 h were recruited to this study.

### **6.3.4 Sample analysis**

The blood samples were analysed in batches at the end of the study period (July 2013). An automated analyser (Sysmex CA-1500 System, Siemens AG, Germany) was used to measure coagulation factor activity ([normal range]: V [60–150]; VIII [52–153], IX [52–156]; VII, X, XI [50–150]) and von Willebrand Factor (vWF) ([50–155]).

### **6.3.5 Automated one-stage factor assays**

The one-stage clotting factor assay was used to identify patients with a deficiency of one or more of their clotting factors. Clinically, this can be demonstrated by prolonged bleeding episodes.

The one-stage clotting factor assay relied on the principle of testing various dilutions of the plasma by either the PT (used for factors II, V, VII and X) or the aPTT (used to assay factors XII, XI, IX and VIII) and comparing the clotting times against a standard curve. A standard curve was generated on the Sysmex using five dilutions of standard human plasma (SHP) in Owren's Buffer, added to an equal volume of factor deficient plasma. The SHP has a known concentration for each factor and the clotting times for each dilution are plotted against the concentration.

The deficient plasma contains all other coagulation factors in normal amounts except the one being assayed for. Therefore, the clotting times were dependent on the quantity of the factor present. Thus, if a patient sample is assayed that has a low fibrinogen, or has more than one factor deficiency, the clotting times will only depend on the factor being assayed. Further details regarding the factor analysis are given in Chapter Two.

### **6.3.6 ROTEM analysis**

Blood samples were processed within one hour of being drawn at 37°C on a ROTEM delta instrument (Pentapharm GmbH, Munich, Germany). All of the treating medical staff were blinded to the ROTEM results. The methodology and the parameters of ROTEM have previously been described in detail ((178), Chapter Two). 20µl of recalcitrant (STARTEM) and 20µl of tissue factor derived from rabbit brain (EXTEM) were placed into the test cuvette, after which 300µl of the blood sample was added. Activation with tissue factor was

performed to standardise the *in vitro* coagulation process and produce a more rapid result. All pipetting steps and the mixing of reagents with samples were performed as standard using the automated electronic pipette program. Clot amplitude at five min (CA5) was reported for each sample analysed in this bleeding subgroup.

### **6.3.7 Data collection**

Data were collected prospectively on patient demographics, time of injury, mechanism (blunt or penetrating), pre-hospital fluid administration, time of arrival at the ED, baseline vital signs and total transfusion requirements in the first 12 h of admission. Injury was classified using the ISS (8). Metabolic acidosis was defined as BD>6mEq/l (194, 195). Coagulopathy has previously been defined as a laboratory PTr>1.2 (36, 64, 194). In trauma, PTr>1.2 has been shown to be a clinically relevant threshold associated with significant increases in mortality and transfusion requirements (64). Normal values for ROTEM clot strength parameters were determined from patients without ATC. It has been shown that ATC is primarily a deficiency in clot strength, not clotting times, and thus, ATC was defined using ROTEM as CA at five min (CA5)  $\leq 35$  mm (75, 124). Results from a cohort of patients with minor injuries (defined by an ISS of  $\leq 4$ ) were used as a baseline comparator as previously described (154). These are referred to as “Minor Injury” in the results section.

### **6.3.8 Outcome measures**

Patients were followed until discharged from hospital or death. Specifically, 24-hour mortality, 28-day mortality, 12-h PRBC requirements and FFP:PRBC ratio measures were recorded.



### 6.3.9 Statistical analysis

Statistical analysis was performed using GraphPad PRISM v5 (GraphPad Software Inc, San Diego, CA, US) and Microsoft Excel 2007 (Microsoft, Inc., Redmond, WA, US). Normal quartile-quartile plots were used to test for normal distribution. Parametric data are expressed as mean  $\pm$  95% confidence intervals (CI) and compared using two-tailed unequal variance Student's t test or one-way ANOVA. Non-parametric data are given as median (interquartile range, IQR). A p value of 0.05 was chosen to represent statistical significance.

## 6.4 Results

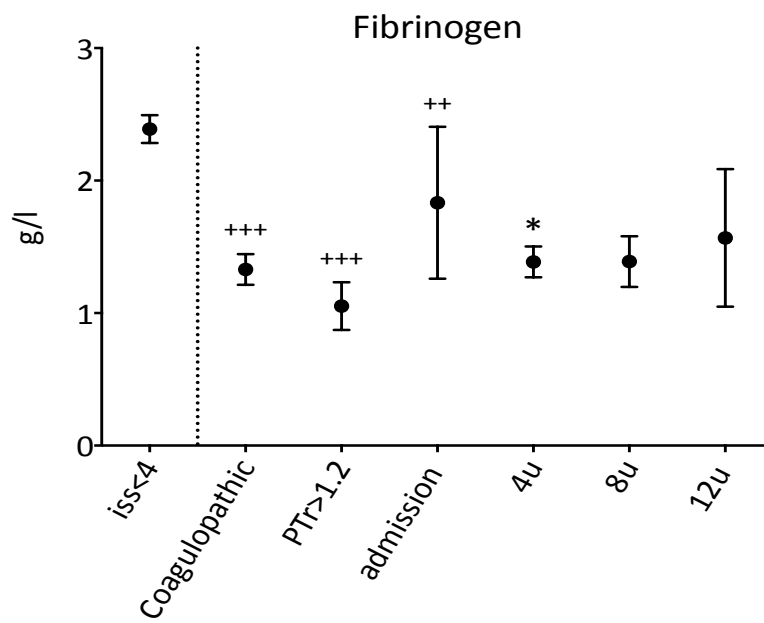
A total of 810 patients were included in the ACIT II study over the 52-month period. Forty-nine individuals were excluded: in such cases, consent was declined or not possible (n=21 patients) or the cases adhered to the retrospective exclusion criteria (n=28). A total of 106 study patients were transfused  $\geq 4$  U PRBCs during the study period, with 27 receiving 8–11 U PRBCs and 31 receiving  $\geq 12$  U PRBCs. The mean FFP:PRBC ratio between intervals was PRBCs 0–4 U: 0.5; PRBCs 5–8 U: 0.9, PRBCs 9–12 U: 0.7 and at h 12: 0.7. Clinical characteristics, admission physiology, and laboratory parameters are detailed in *Table 6.1*.

<b>Patients</b>	<b>Minor Injury</b>	<b>Bleeding</b>
<b>Number</b>	139	106
<b>Age</b>	33 (22–42)	44 (30–60)*
<b>Male</b>	116 (83%)	75 (76%)
<b>Injury</b>		
ISS	1 (1–4)	34 (25–41)*
ISS>15	0	99 (94%)*
Blunt injury	100 (72%)	92 (87%)*
<b>Admission parameters</b>		
SBP<90 mmHg	5 (4%)	41(46%)*
BD>6 mEq/L	7 (5%)	68 (67%)*
PT (secs)	10.9 (10.5–11.3)	12.9 (11.7–14.6)*
PTtr>1.2	2 (2%)	40 (40%)*
CA5≤35 mm	12 (9%)	41 (43%)*
<b>Fluid requirements (first 12 h)</b>		
Pre-hospital PRBCs (units)	0(0)	0 (1–6) *
Pre-hospital Crystalloid (ml)	0(0–100)	500(100–2,000)*
Crystalloid (ml)	0 (0–800)	2,500 (1,300–4,000)*
Colloid (ml)	0 (0)	875 (0–1,688)*
PRBCs (units)	0 (0)	8 (5–13)*
PRBCs≥10units	0 (0)	40 (38%)*
FFP (units)	0 (0)	6 (4–8)*
Platelet (units)	0 (0)	1 (1–2)*
Cryoprecipitate (units)	0 (0)	2 (0–2)*

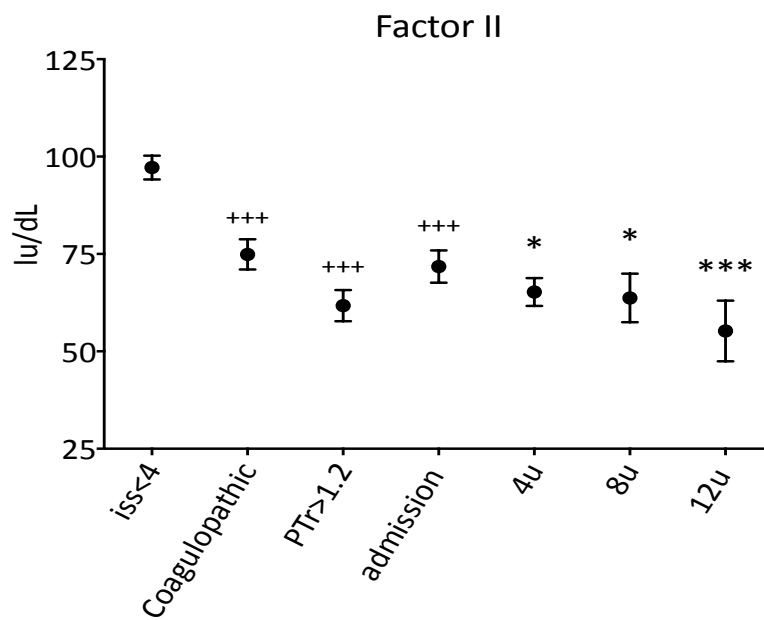
**Table 6.1. Patient demographics and clinical characteristics.** *Values are given as n (%) or median (interquartile range).*

The evolution of coagulation factor concentration during haemorrhage was evaluated (*Figure 6.1A–J*). On admission, the 106 bleeding trauma patients had an almost universal deterioration in coagulation factor concentration when compared to the minor injury group ( $p<0.05$ ). By far the greatest reduction was seen in factor V activity (reduction of 45%,  $p<0.001$ , *Figure 6.2C*). However, vW:Ag showed a 46% increase in circulating concentration ( $p<0.001$ ) and factor VIII concentration remained virtually unchanged. On admission to the ED, 16% of bleeding patients had at least one critically low factor level when compared to standard ranges ( $<50\text{IU/dL}$ ). This increased to 19%, 26% and 33% for 4 U, 8 U and 12 U PRBCs respectively.

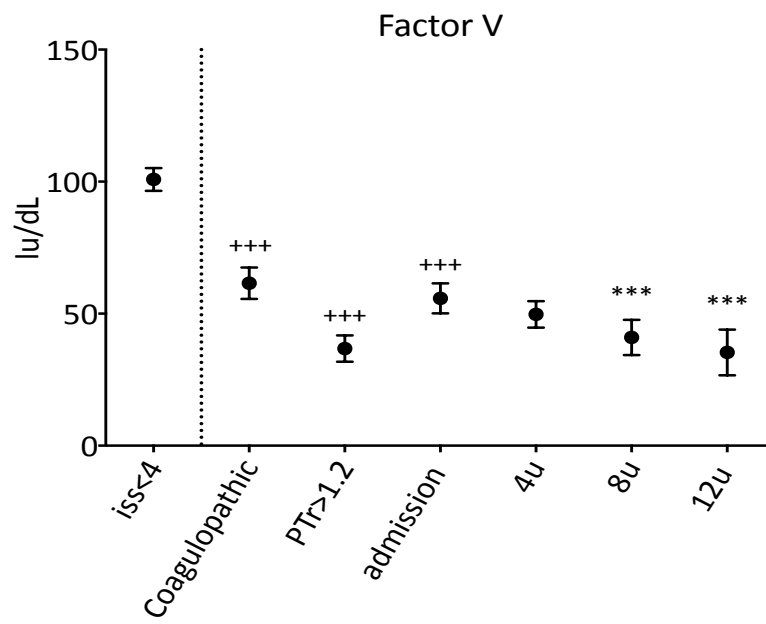
As trauma patients continued to bleed, there was a gradual deterioration in coagulation factor concentration as compared to the minor injury group. By the end of the 4th U PRBCs, all the coagulation factor levels were below 70% of the minor injury cohort, with the exceptions of vW:ag and VII. At 4 U PRBCs, the average factor activity was 70% when compared to standard ranges (excluding VIII and vW:Ag). As bleeding persisted, by the 8th U PRBCs, the average factor activity fell to 56%. At the point of the 12th PRBC transfusion, there was a further reduction with an average factor concentration of 50% as compared to standard ranges. Patients who required 12 U PRBCs showed the greatest reduction in factors II, V, VIII, IX and X when compared to admission levels ( $p<0.05$ ). Again, the greatest fall in activity was seen in factors V (35%) and VIII (36%) as compared to the minor injury cohort. However, fibrinogen concentration showed a significant trend towards admission concentration. Overall, a deterioration in coagulation factor concentrations as the patients bled could be observed. Nevertheless, while the reduction of coagulation factor concentrations reached statistical significance, only factor V fell to a clinically significant level. The remainder were mostly within laboratory-reference ranges (0.5–1.5 U/mL). In summary, DCR was able to maintain coagulation factor levels when compared to admission, but did not return levels to minor injury levels.



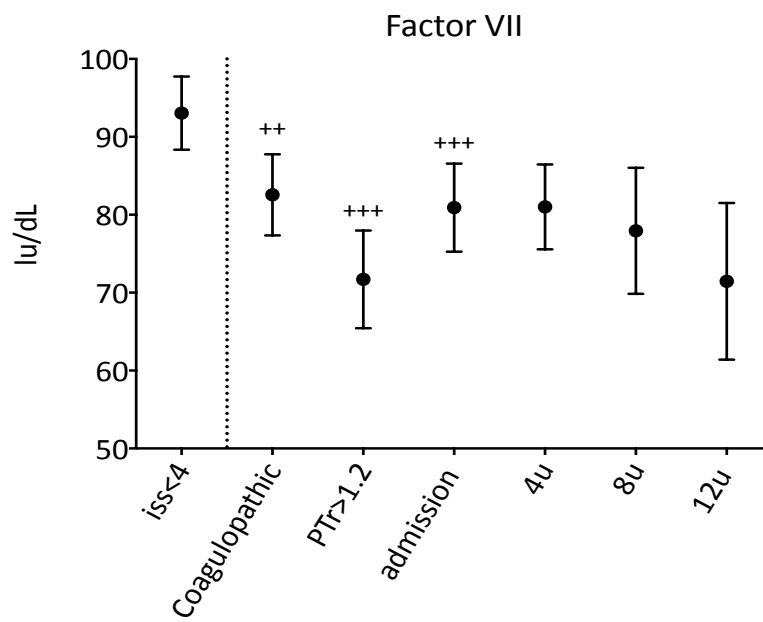
**Figure 6.1A.**



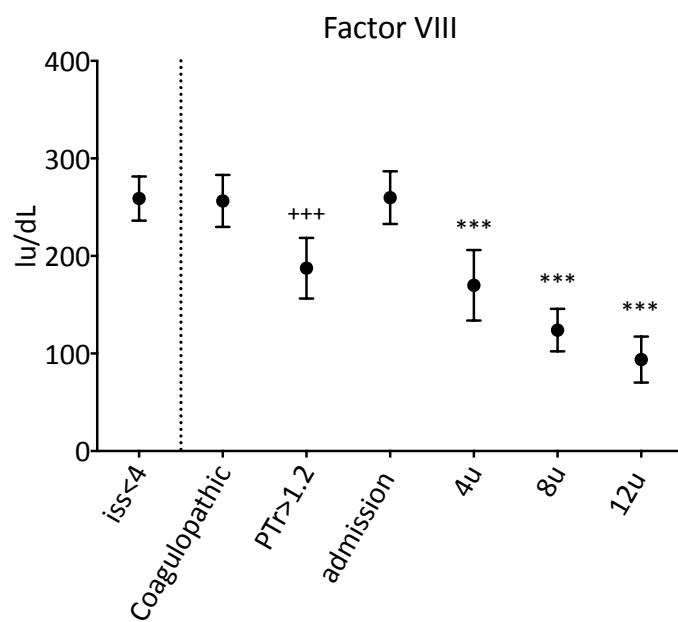
**Figure 6.1B.**



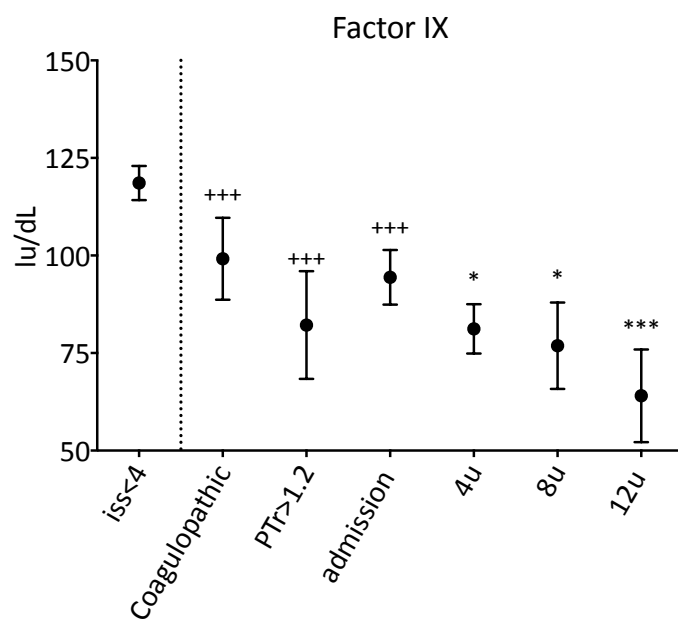
**Figure 6.1C.**



**Figure 6.1D.**



**Figure 6.1E.**



**Figure 6.1F.**

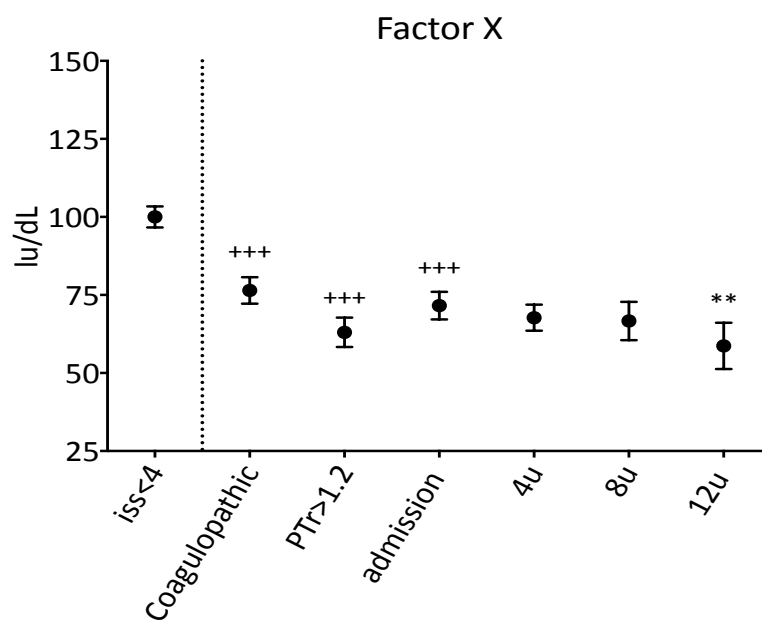


Figure 6.1G.

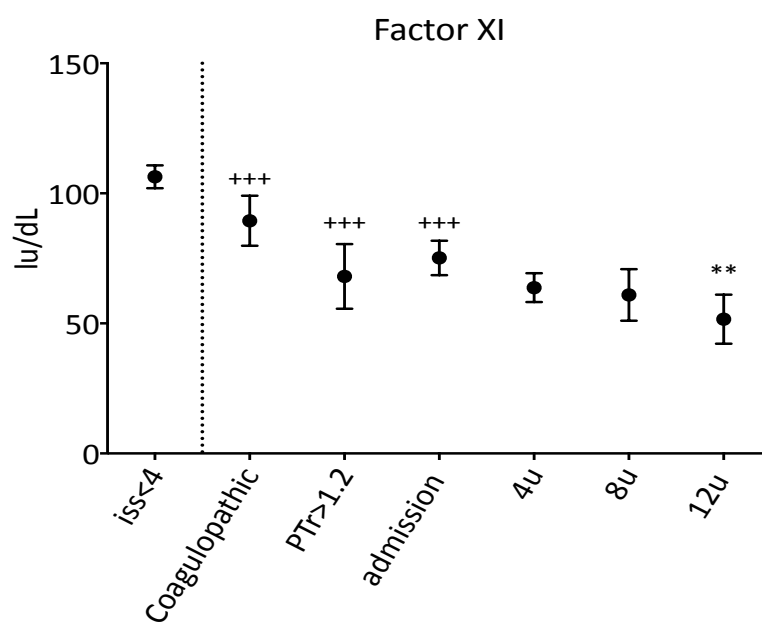


Figure 6.1H.

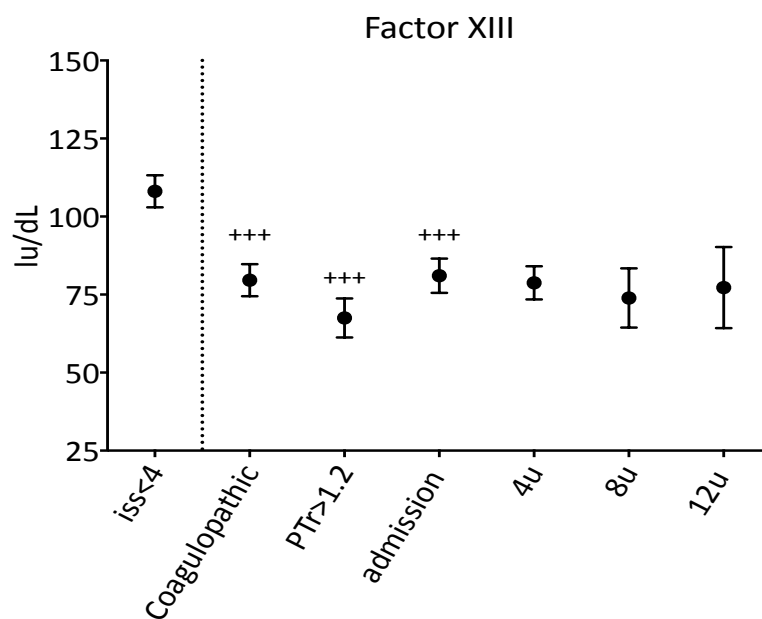


Figure 6.1I.

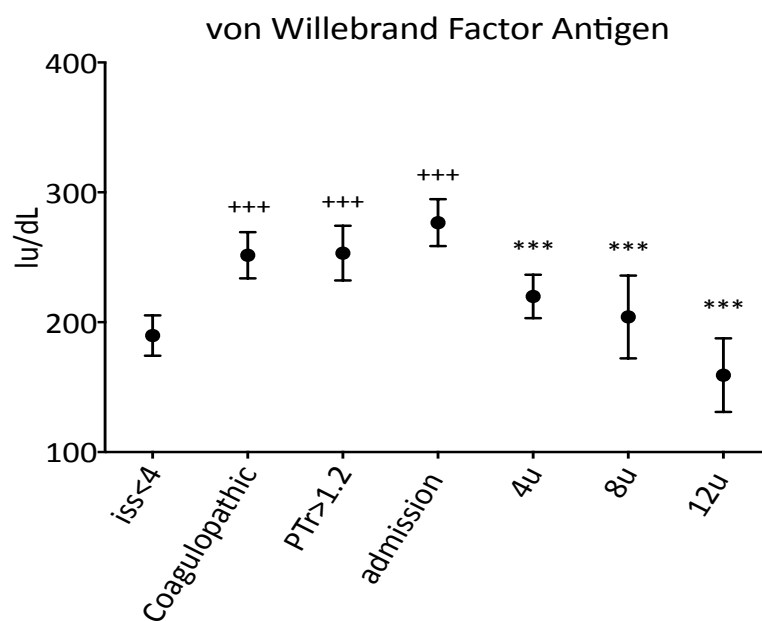
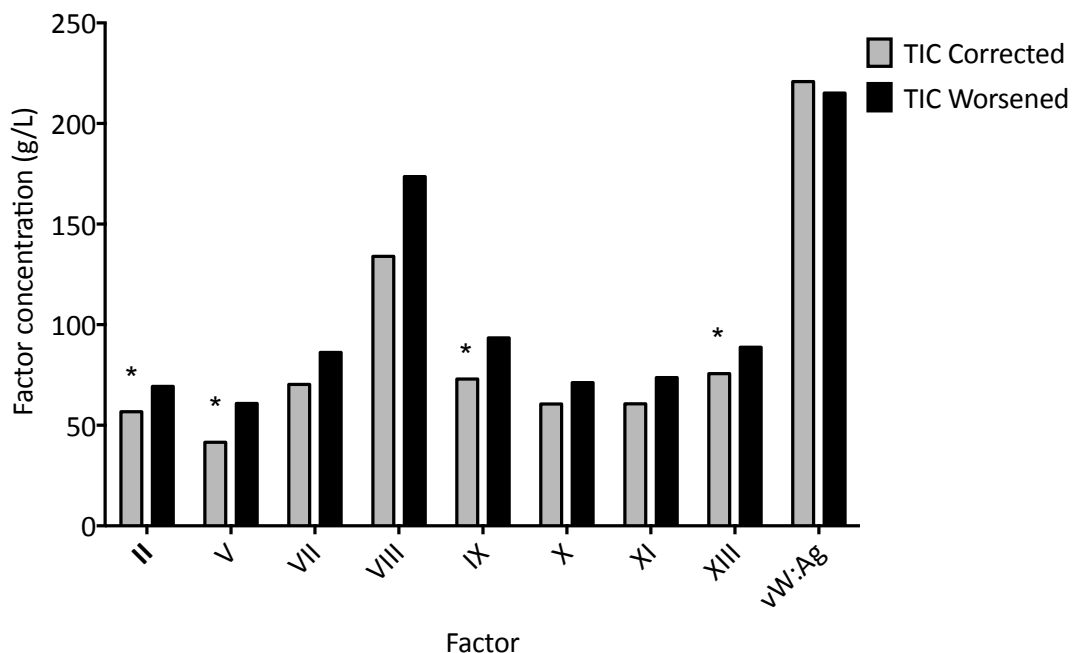


Figure 6.1J.

**Figure 6.1A–J. The effect of DCR on coagulation factor concentration in actively bleeding trauma patients.** *Coagulopathic, PT>1.2 and admission were compared to ISS<4 (+  $p<0.05$ ). 4 U, 8 U and 12 U are compared to admission (\* $p<0.05$ ).*



Next, the effect of TIC on coagulation factor concentrations was examined (*Figure 6.1*). When comparing coagulopathic patients to the minor injury group, it was noticed that there was a statistically significant deterioration in all coagulation proteases ( $p<0.05$ ). Those patients with an admission of  $\text{PTTr}>1.2$  were still worse ( $p<0.05$ ). However, although there was a statistically significant deterioration in concentration, none of the coagulation proteases reached values that were clotting factor activity level  $\leq 30\%$  of normal reference ranges. Patients who corrected their TIC were compared against those who became coagulopathic after DCR (*Figure 6.2*). Among those patients that corrected their TIC, there was an almost universal decrease in absolute values of coagulation factor levels. The greatest difference seen was in factors II, V, IX, and XIII. In both groups, however, none of the coagulation factors reached clinically critical levels of 30% below normal laboratory values.



**Figure 6.2. Coagulation factor concentration in those patients who had their TIC corrected compared to those patients who worsened during the bleeding phase (\*  $p<0.05$ ).** Overall, the majority of coagulation proteases were found to be in lower concentrations in patients who corrected their TIC. Factors II, V, IX and XIII had statistically significant lower concentrations in those patients who corrected their TIC ( $p<0.05$ ).

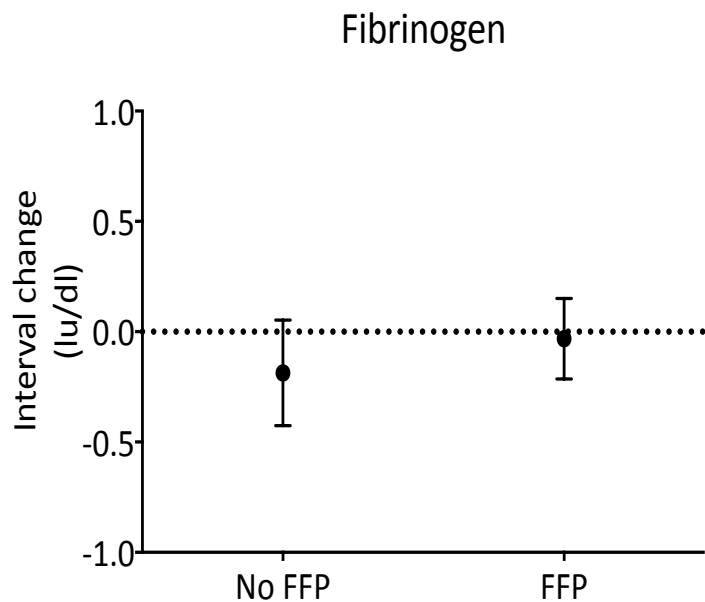
I examined the effect of FFP transfusion on coagulation factor concentration. On average, transfusion of FFP had little effect on coagulation factor concentration (*Figure 6.3A–J*). When broadly examining those patients who received FFP with those who did not receive any, we did not find any statistically significant improvement in the interval change in coagulation factor concentration. On average, there were minor improvements in interval change in all coagulation proteases apart from factor VIII and vW:Ag, although none reached statistical significance. Next, I investigated the effect of increasing doses of FFP on coagulation factor interval change (*Table 6.2*). On average, FFP in PRBC ratios of 1:2 or higher generally preserved levels procoagulant coagulation factors II, V, IX, X, XI and XIII. However, the difference in these factor levels between low and high FFP ratios was small, varying by only 5–10% of the baseline (*Table 6.2*). Transfusing higher ratios of FFP did not confer any additional benefit over lower ratios in correcting coagulation factor concentration.

There was a varied effect on coagulation factor concentration regardless of type of blood component administered. I examined the effect of blood component use on the interval change in coagulation factor concentrations in bleeding patients (*Table 6.2*). The greatest deterioration in overall coagulation factor concentration was seen when transfusing PLT in addition to CRYO and FFP. A moderate improvement in interval change in coagulation factor levels was seen in V, IX and X when transfusing FFP and CRYO. Overall, however, there was little change seen in coagulation factor concentration regardless of the blood component used.

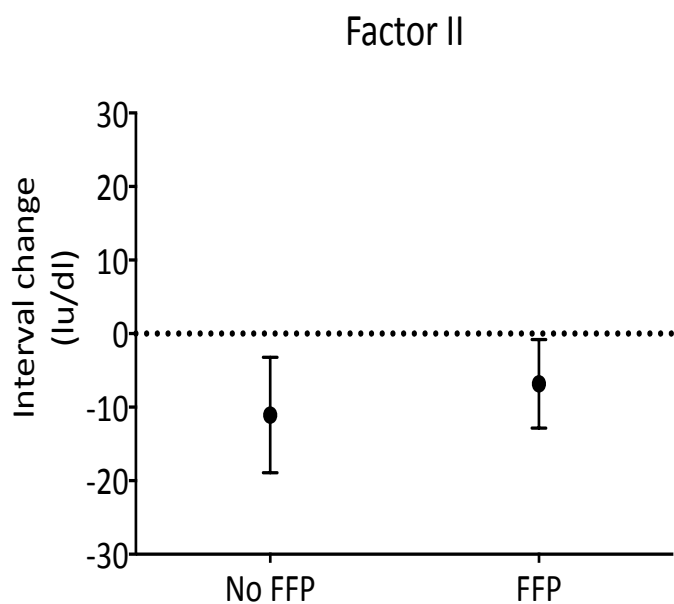
Factor	Absolute Values		Average Interval Change					
	Minor Injury	Admission	≤1:4	1:2-3:4	≥1:1	PRBC only	FFP +CRYO	FFP+CRYO+PLT
Fibrinogen (g/L)	2.4	1.8	-0.2	-0.1	0.0	-0.2	-0.1	0.1
II (Iu/dL)	97.2	71.8	-10.8	-8.2	-5.1	-10.3	-6.2	-10.7
V (Iu/dL)	100.8	55.8	-11.2	-12.0	-3.2	-8.6	-8.3	-7.9
VII (Iu/dL)	93.1	80.9	-3.2	-0.7	1.3	-4.2	0.4	-3.7
VIII (Iu/dL)	258.9	259.8	-87.8	-98.2	-83.6	-73.1	-102.6	-109.6
IX (Iu/dL)	118.6	94.4	-16.4	-8.9	-10.5	-16.1	-9.2	-11.4
X (Iu/dL)	100.0	71.6	-7.9	-7.0	-1.5	-7.2	-3.4	-9.5
XI (Iu/dL)	106.4	75.2	-13.2	-3.7	-10.8	-13.7	-7.0	-12.1
XIII (Iu/dL)	108.1	81.1	-11.2	-4.1	2.2	-8.0	-1.9	-3.9
yW:Ag (Iu/dL)	189.7	276.7	-52.9	-44.5	-60.0	-58.3	-54.5	-88.2

**Table 6.2. Effect of blood component therapy on coagulation factor concentration when administered during DCR (mean).** \* $p<0.05$  signifies coagulation factor level comparison to admission only. Coagulation factor concentration was also compared to increasing FFP:PRBC ratios. There was no significant improvement in any procoagulant factor with an increasing FFP:PRBC ratio transfused. Blood component therapy was compared to PRBCs alone (\* $p<0.05$ ). Minor injury was used a visual comparator

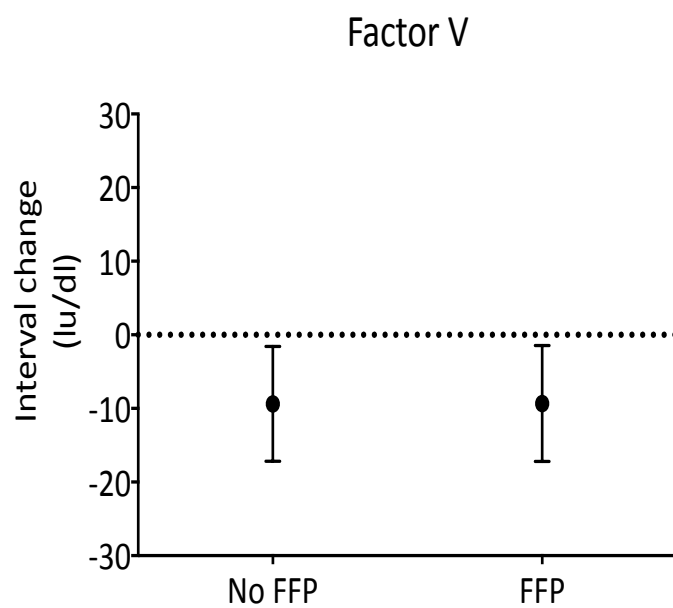
**Figure 6.3. The effect of a FFP transfusion on coagulation factor concentration in bleeding patients.**



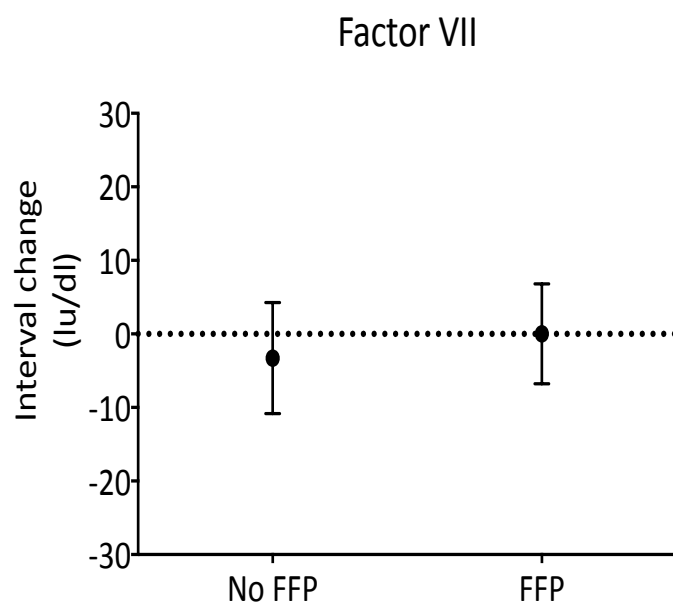
**Figure 6.3A.**



**Figure 6.4B.**



**Figure 6.4C.**



**Figure 6.4D.**

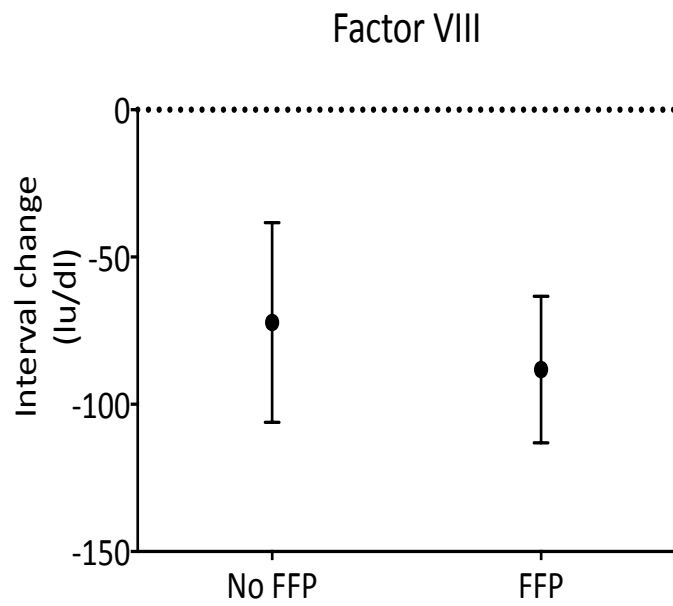


Figure 6.4E.

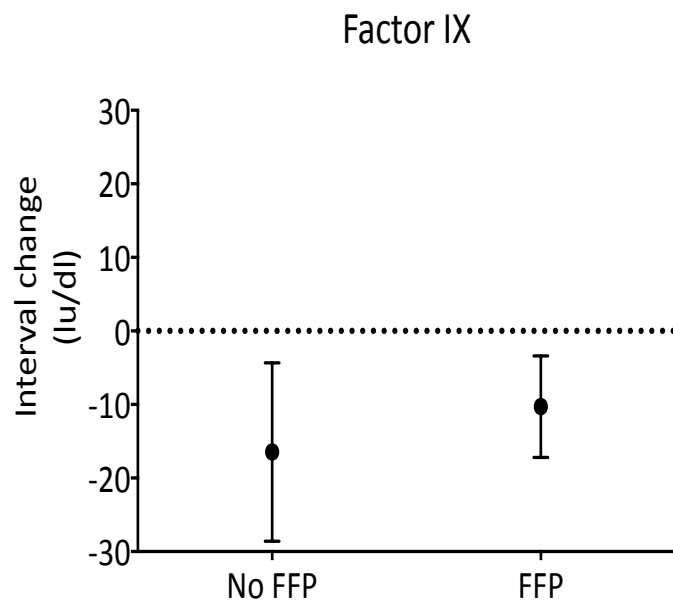
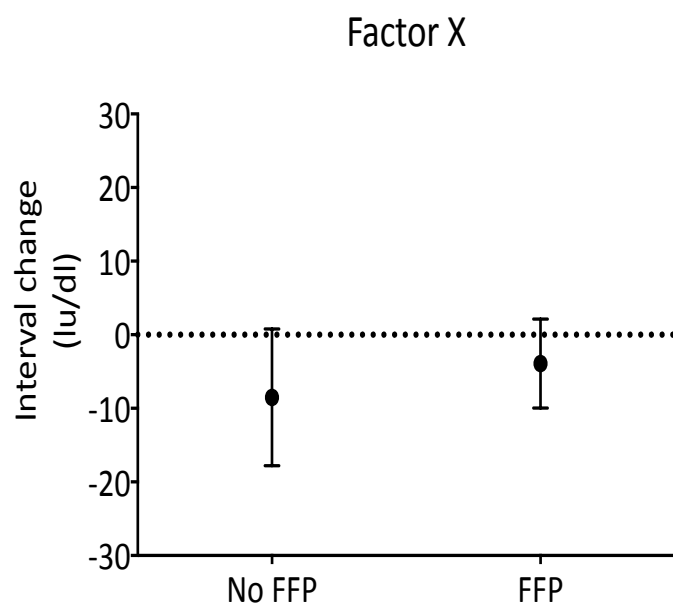
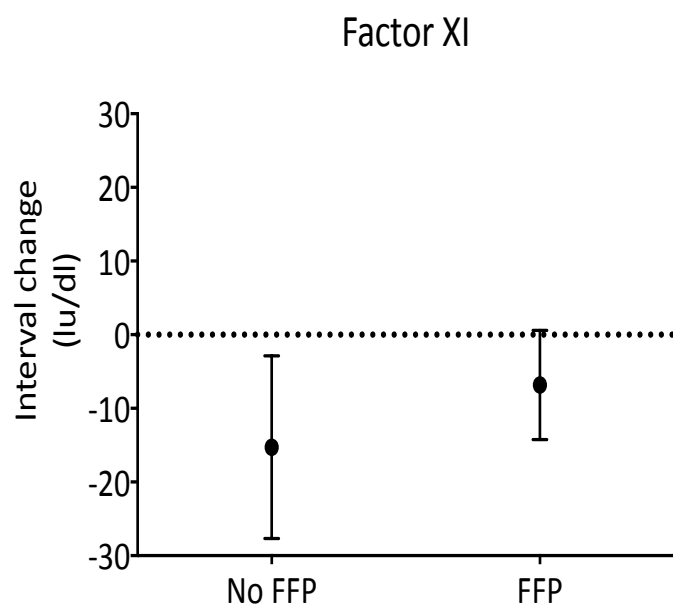


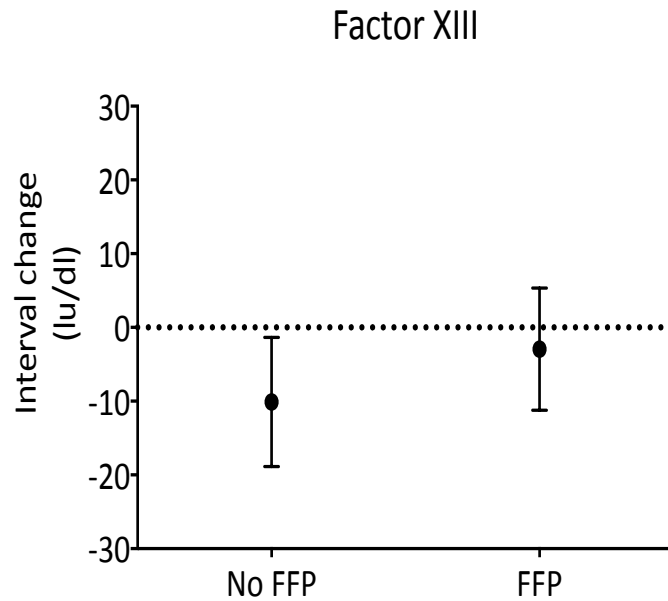
Figure 6.4F.



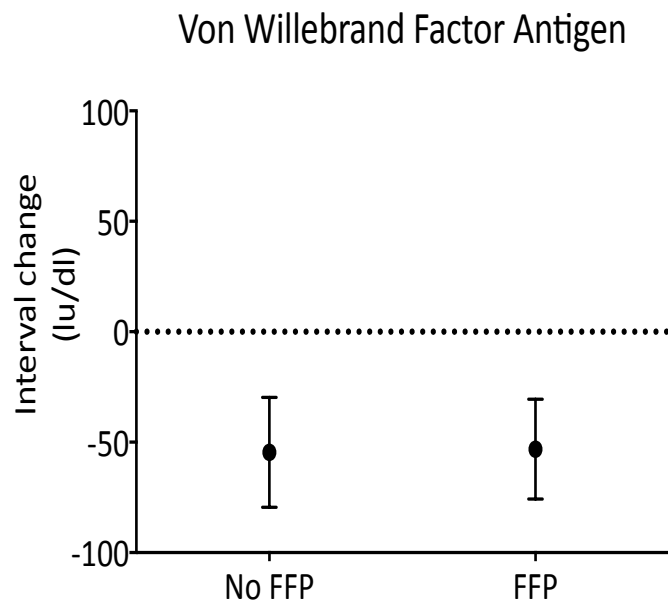
**Figure 6.4G.**



**Figure 6.4H.**



**Figure 6.4I.**



**Figure 6.4J.**

**Figure 6.3A–J. The effect of FFP transfusion on coagulation factor concentration in bleeding patients.** Values show interval change in coagulation factor concentration. In general, there was a decreased interval change in coagulation factor concentration in those patients that did not receive FFP, but this did not reach statistical significance. (\* $p < 0.05$ ).



## 6.5 Discussion

In this study, the effects of DCR on coagulation factor concentrations in severely injured trauma patients were prospectively examined. The majority of bleeding trauma patients had a statistically significant deterioration in coagulation factor concentration on admission. These levels continued to fall as patients bled, although by the 12th U, only factor V activity decreased to *clinically* relevant concentrations. Patients who successfully corrected their TIC by DCR techniques had, on average, lower coagulation factor levels than those that went on to develop TIC, although no single coagulation protease fell below critical threshold for normal functional activity. Furthermore, this work revealed that transfusions of FFP in average doses had little effect on coagulation factor concentration and transfusing higher ratios of FFP did not confer any additional benefit over lower ratios in correcting coagulation factor levels. Overall, blood component therapy had little discernable effect on coagulation factor concentration.

In the severely injured trauma patient, TIC represents a significant contribution to death. It is vital that prompt and appropriate haemostatic intervention is implemented to prevent and correct life-threatening bleeding. One approach proposed for preventing exsanguination and promoting haemostatic control is to transfuse patients with a fixed FFP:PRBC ratio, in addition to CRYO and platelets, in order to provide optimal concentrations of coagulation factors (88, 103, 176). However, little research has been undertaken in either understanding the evolution of coagulation factor concentration in actively bleeding trauma patients or the effect of these blood components on coagulation factor activity.

Historically, it is thought that coagulopathy was the result of an ongoing cycle of dilution and consumption of coagulation factors, hypothermia and acidosis (121). A

previous small case-control study of trauma patients with (n=38) and without (n=53) TIC, conducted by Shaz et al., reported that following injury, TIC is associated with decreased factor activities (196). However, in contrast with findings reported in this chapter, Shaz et al.'s TIC group were less severely injured (mean ISS 14 vs 34) and infused with greater volumes of pre-hospital crystalloid (852 ml vs 500 ml). Furthermore, only a single time point of coagulation factor level (admission) was analysed. In contrast, this study's multiple time point analysis demonstrated that the majority of coagulation factors do not deteriorate to below critical levels. Previous studies have investigated the relationship between FFP:PRBCs and mortality proposed through increased coagulation factor concentration (121).

The results reported in this chapter show that, on average, patients who present with TIC do not have critical levels of coagulation factor activity. Furthermore, transfused higher ratios of FFP:PRBCs do not, on the whole, improve coagulation factor concentrations. Therefore, the combination's haemostatic benefits may be through alternative mechanisms other than solely improving coagulation factor concentration. Moreover, the greatest benefit in product transfusion was seen in the combination of FFP and CRYO and, in contrast to current opinion, there was no significant improvement of interval coagulation factor activity when adding platelets.

The clinical significance of minor derangements in coagulation factor activity is uncertain. Jansen et al. performed a post hoc analysis of the effect of hypoperfusion (by measuring lactic acidosis) on the activity of coagulation factors (190, 197, 198). This analysis concluded that hypoperfusion in trauma patients was associated with a moderate, dose-dependent reduction in the activity of coagulation factors II, VII, IX, X, and XI, and a more marked reduction in factor V activity, which is relatively independent of the severity of shock. Moreover, they found preservation of coagulation factor activity in the majority of normally and moderately hypoperfused patients. This

suggests that aggressive administration of plasma is probably only indicated in severely hypoperfused patients. The results presented in this chapter support these findings and also that of Rizoli et al. with the greatest deterioration seen in factor V as patients continue to bleed (192). Despite this, the majority of patients in both studies had coagulation factor activity levels within the normal range, implying that either the reference ranges for trauma patients may need to be adjusted to represent the injured population or, alternatively, minor alterations in coagulation factor activity have possibly resulted in a combined added effect, resulting in altered haemostasis (192). Their study, like others (192), was limited to using critical coagulation factor activity levels associated with clinically relevant abnormalities. These thresholds are based on the study of patients with hereditary *single-factor* deficiencies which may not be applicable to the trauma setting. Furthermore, it is classically accepted that the loss of 70% of coagulation factor concentration is not generally associated with a bleeding diathesis (199). It is below this level, with approximately 30% residual coagulation factors, that the PTr is approximately 1.5 times the midpoint of the normal reference range, levels that should provide sufficient functional clotting factor activity (199). This is broadly accepted as indicative of a major haemostatic defect and has been used by haematologists as the threshold for normal blood clotting in congenital coagulopathies. However, TIC is an acquired coagulopathy and previously published work has shown that clinically relevant coagulopathy is as prevalent at PTr values of greater than 1.2. Therefore, while in non-trauma patients, normal haemostasis requires only 30% of activity from each individual clotting factor, the levels needed after injury or when multiple deficiencies coexist are not known. Furthermore, it is possible that they are significantly higher than the critical thresholds used in this study. Therefore, the critical threshold for coagulation factor activity is perhaps greater than 30% and should be re-evaluate using minor severity injured trauma patients as a comparator.

## **6.6 Limitations**

This study has several limitations. As described above, there are inherent problems with using a threshold value of 30% for defining a critical clotting factor deficiency—this definition has not previously been used in the trauma context. It was impossible to perform coagulation factor assays in all bleeding patients, mostly due to difficulties in obtaining sufficient blood samples in all patients. This issue was predominantly seen in patients that exsanguinated at a high rate and therefore sequential blood draws were logistically impossible. Another limitation was the lack of standardised definitions for early trauma-associated coagulopathy. There is no universally acknowledged threshold value of INR or aPTT that defines coagulopathy, potentially affecting the translation of these findings.

## **6.7 Conclusions**

The findings of this study have important clinical implications. The pillars of DCR are aggressive clotting factor replacement with permissive hypotension. Early high-dose FFP elicits a variable response in the coagulation system. It is long believed that blood component therapy exerts its haemostatic benefits via increased concentrations of active coagulation factors. The present study shows that, on the whole, critical clotting factor deficiencies do not occur immediately after injury, prior to substantial fluid resuscitation.

## CHAPTER SEVEN

# **Conclusions**

## **7.1 Summary of the findings**

This thesis has tested the hypotheses described in Chapter One, and has led to significant and novel contributions to the body of knowledge regarding the role of blood component use in DCR.

Chapter Three successfully described the effect of the implementation of an MHP on outcome and blood product administration. Major haemorrhage protocols are required as part of DCR regimens in modern trauma care, and the hypothesis tested in this study stated that major haemorrhage protocols improve blood product administration and reduce waste compared to traditional MTPs.

Fundamentally, outdated MTPs targeted dilutional coagulopathy that occurs after a massive transfusion, while MHPs target ATC. This approach fundamentally supports a new way of thinking, moving from identifying patients who will, at some point, require 10 or more units of blood, to identifying those who, on arrival, are bleeding and already coagulopathic. I believe that a MHP is therefore conceptually more suited to the DCR paradigm.

In this study, the administration of blood component therapy significantly improved post MHP implementation, with specific enhancements to both FFP and CRYO to PRBC ratios. Platelet transfusion improved by 15%, and there was a significant reduction in the wastage of platelets from 14% to 2%. The introduction of an MHP also correlated with improved clinical outcomes, where median hospital LOS was reduced by half. In conclusion, implementation of a MHP results in improved delivery of blood components and a reduction in the wastage of blood products compared to the older model of a MTP. Furthermore, in combination with educational programmes, MHPs can significantly

improve blood product administration and patient outcomes in trauma haemorrhage as well as reduce cost.

Chapter Four hypothesised that TIC deteriorates during acute trauma haemorrhage. Within this study, I have successfully described the effects of DCR in the acute phase of trauma haemorrhage. The evolution of coagulopathy and the effect of DCR in correcting TIC and restoring tissue perfusion during acute haemorrhage had not been previously studied in the context of severely injured trauma patients. The specific aims of the chapter were to characterise the incidence and severity of TIC during the acute phase of DCR, characterise lactate clearance during haemorrhage, and determine whether DCR achieves its stated aims of correcting coagulopathy and restoring perfusion during trauma haemorrhage.

I conducted a prospective cohort study of ROTEM and lactate measurements taken from trauma patients. On admission, 40% of patients were coagulopathic and this number increased to 58% after 4 U of PRBCs and to 81% after 8 U of PRBCs. On average, all functional coagulation parameters deteriorated during haemorrhage. Clot strength and clotting times only corrected when no further PRBCs were needed. The average admission lactate was 6.2 mEq/L. Trauma patients with hyperlactatemia on admission did not clear lactate until haemorrhage control was achieved and no further transfusion of PRBC units was required. I concluded that DCR offers several advantages over traditional strategies; however, it still does not achieve the correction of hypoperfusion or coagulopathy during the acute phase of trauma haemorrhage.

Chapter Five succeeded in determining the efficacy of blood component therapy in the correction of TIC during haemorrhage. DCR strategies target TIC with the early delivery of high-dose blood components such as FFP and platelet transfusions. However, the

ability of these products to correct TIC during haemorrhage and their effect on the coagulation profile was unknown.

Within this study, I demonstrated that there was no clear benefit observed with high-dose FFP therapy in any coagulation parameter, contrary to current belief. Only combined high-dose FFP, CRYO and platelet therapy, along with a high total fibrinogen load, appeared to produce a consistent improvement in coagulation. This chapter concluded that DCR with standard or average doses of blood components does not consistently correct TIC during haemorrhage.

The importance of the findings of both Chapter Four and Chapter Five are particularly relevant in light of the recent publication of the Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial (138). This multicentre, randomised and controlled trial suggests better outcomes from a 1:1:1 ratio in secondary analyses. In contrast to the data presented in this current thesis, the authors of the PROPPR trial did not present any data on the effect of the different ratios on coagulation or resuscitative parameters. At present, it is therefore unclear how a higher dose of plasma might exert its effect on haemostasis.

In Chapter Five, I was able to expand on the relationship between blood component delivery during the acute phase of trauma haemorrhage and the viscoelastic measures of coagulation. In both Chapter Four and Chapter Five, the majority of major haemorrhage protocols were activated in the field by physician-led medical teams. Consequently, the first products transfused on arrival were PRBCs and FFP in high empiric ratios with pooled platelets (equivalent to 6 U) administered in the second and subsequent packs or coolers. All of the study patients were treated at Level 1 trauma centres with 24-hour access to damage control surgery with anatomic control of haemorrhaging. Completing



transfusions was therefore rapid and typically occurred within the first two to three hours following admission, thus allowing the analysis of the acute bleeding phase.

The PROMMTT study has also provided valuable observational data on trauma-induced coagulation, transfusion, and outcome (56, 200). Restricted crystalloid use remains a key component of hemostatic resuscitation; however, neither the PROMMTT publication nor the PROPPR study presents any data on crystalloid use (138, 200). Cotton et al. (185) have shown, in a contemporary retrospective study of DCR versus pre-DCR, that crystalloid use, although reduced in DCR, remains not insignificant, with equivalent volumes (pre-hospital, 300–900 mL; emergency department, 200–2,000 mL; and operating room, 1,800–4,100 mL) to those reported in this thesis. Moreover, another publication from the PROMMTT study has actually demonstrated that pre-hospital crystalloid use is associated with *improved* survival (201). In both of my studies, none of the patients received colloid during active haemorrhage. The question of optimal intravenous fluid administration in the early phase that follows the control of major haemorrhage remains unanswered, particularly in patients who continue to be intravascularly depleted without signs of ongoing bleeding and adequate haemoglobin.

The primary focus of these two chapters was to examine whether hemostatic resuscitation is able to correct coagulation, restore tissue oxygenation, and repay oxygen debt in trauma haemorrhage. I have shown that DCR with standard doses of blood components does not consistently correct TIC during ongoing bleeding.

In this body of work, I have shown that the mean dose of FFP administered (3.2 U) per 4 U of PRBCs has no effect on clotting time, clot generation, or maximum clot firmness on ROTEM. Adding cryoprecipitate (an average of 1.6 pools per interval) and platelets (an average of 1 pool per interval) showed some protection of the clotting function across the interval, but only an improvement in the clotting time was statistically

significant. Moreover, there was no clear benefit connected to high-dose FFP therapy in any coagulation parameter. It seems that the magnitude of coagulopathy is so large that, in trauma patients with uncontrolled haemorrhage, restoration of normal clotting factor levels may not be possible with current transfusion algorithms that use products less concentrated than whole blood. Further research is required on the efficacy of the early, concentrated, and targeted replacement of key clotting substrates—for example, fibrinogen in not only reversal of coagulopathy but also for controlling haemorrhage and in terms of patient outcomes.

Finally, Chapter Six successfully described the effect of blood component therapy on coagulation factor concentration during trauma haemorrhage.

Given that a mainstay of trauma management is the replacement of lost and consumed coagulation factors, the very fact that coagulopathy is commonly seen during trauma resuscitation points to a possible hypothesis that we are not giving enough coagulation factors during this phase of care. Many classical accounts of TIC describe it as a relatively late event resulting from consumption and loss of clotting elements and their iatrogenic dilution by resuscitation (32). Consequently, aggressive transfusion therapy with FFP and platelets could prevent or correct clotting deficiencies by providing higher concentrations of coagulation factors, and may improve the coagulation profile and survival.

As stated above, the pillars of DCR include aggressive clotting factor replacement with permissive hypotension. However, the principle findings of Chapter Six showed that not only were coagulation factor concentrations, on the whole, maintained on admission, but they also did not fall to clinically critical levels in severely injured and bleeding trauma patients. Interestingly, investigators from the PROMMTT study also conducted a subset analysis of 165 trauma patients, where plasma was prospectively assayed for coagulation

factor concentration (202). In this subset analysis of TIC vs non-TIC patients, TIC was shown to be characterised by reductions of coagulation proteases and fibrinogen; however, none of these coagulation proteases reached *clinically significant* concentrations. This data, in addition to the work presented in Chapter Six, gives weight to the argument that TIC may be mediated by the activation of the protein C system (75).

Activated protein C is a serine protease, which, once activated in a mechanism that involves thrombin, thrombomodulin, and the endothelial protein C receptor, mediates its anticoagulant effects through direct proteolytic cleavage of factors V and VIII. The data presented here—in addition to the data generated by the PROMMTT study—showed decreased factor V to a point of clinically relevant concentrations and a trend towards significance in factor VIII levels, which lends support to the aPC hypothesis that ATC is mediated through the proteolytic inactivation of factors V and VIII. In a separate study, Rizoli et al. also reported that 20% of all severely injured patients had critical clotting factor deficiency on admission, particularly of factor V, which indicates a broad depletion of factor levels after injury (192) and is suggestive of coagulopathy after trauma. However, the majority of patients in this study still had coagulation factor activity higher than critical concentration reference ranges.

Furthermore, findings from Chapter Six revealed that transfusions of blood components in the form of FFP in average doses had little effect on coagulation factor concentrations. Moreover, transfusing higher ratios of FFP did not confer any additional benefit over lower ratios in terms of correcting coagulation factor levels. It remains unclear whether reduced factor levels in general represent enhanced but nonpathologic functional protease activation toward fibrin production, or whether these reduced levels seen here and in studies published by others is evidence of dilution or pathologic

depletion. Nonetheless, blood component therapy had little discernable effect on coagulation factor concentration.

## **7.2 Strengths and limitations of this thesis**

A significant achievement of this project is to have set up and conducted the UK's single largest ongoing prospective evaluation of blood component therapy in trauma patients to date. Although it is notoriously difficult to undertake and traditionally prohibitive to conduct research in trauma, a further key strength of my study is the successful use of emergency consenting procedures. Specifically, the use of a PrLAR was able to provide consent for inclusion in the ACIT II study. Patient consent was successful in over 95% of cases, thus demonstrating that large-scale research can be performed in the emergency clinical scenario through the application of a well-designed and executed protocol. The enrolment of all patients requiring trauma team activation provided a control population of trauma patients with only minor injuries (ISS<5) and normal admission physiology. The large number of patients enrolled enabled normal reference ranges for all parameters to be established for this uninjured cohort. This robust system remains in place, and thus enables ongoing data collection. This is the first prospective study to show evidence that aggressive transfusions of plasma have very little effect in reversing the coagulopathy caused by severe trauma bleeding.

There are, however, notable limitations to the work presented in this dissertation. In the first instance, the study described in Chapter Three could have been subject to inaccurate documentation of patient characteristics and their outcomes due to the study's retrospective nature. However, despite historic controls, there is a relatively tight time frame between the two studied periods and the clinical characteristics of the two cohorts, and presenting physiology was very similar.

Furthermore, the triggering criteria for the MHP had not been validated, although clinically relevant and based on expert consensus. The clinical assessment of fluid response is, of course, vital to the assessment of ongoing haemorrhage, as opposed to a single blood pressure reading. The clinician must also suspect haemorrhage rather than tamponade or other shock physiology. Unfortunately, the exact timing of plasma administration was not available to me in this retrospective study, and it is now the subject of my ongoing prospective work. Consequently, without continuous assessment of coagulation, it is difficult to know when coagulopathy was actually resolved; therefore, it was not possible to examine this in Chapter Three, but, again, I explored this prospectively in later chapters.

The trauma population studied in this thesis are a heterogeneous group. I enrolled patients known to have, or who have had, a high index of suspicion for bleeding based on abnormal primary survey findings at admission, as, at this stage, one is unable to detect the exact nature of their injuries in order to match injury cohorts. While this resulted in a heterogeneous cohort, my study does reflect the clinical presentation of polytrauma patients as seen in MTCs and for whom MHPs and targeted component therapy may have the most utility.

I found a large variability in the coagulation response to FFP transfusion among the different study participants. In order to adequately account for this heterogeneity in future studies, I will need to use data mining techniques and exploratory data analysis to understand the relevance of each variable, and to dimensionally reduce the complexity. Techniques that could be used include clustering, principal component analysis and network analysis. These techniques would help to understand the patterns in larger data sets, to reduce the variables to those that are mathematically most relevant, and to explore the data in ways that are not limited to traditional scrutiny.

Collecting samples from bleeding patients was, in some cases, particularly challenging, especially across three international centres. In order to deal with the complexities of an international observational study, regular site visits to audit data entry were carried out. This not only ensured that the strict inclusion criteria for the ACIT II study was adhered to, but also enhanced the accuracy of the data analysed.

Missing data for blood samples were, unfortunately, frequent, due in part to the practical difficulties involved in taking blood from patients with such critical injuries. This occurred over all three sites. Reliance on pre-hospital data was kept to a minimum, however, as all three centres collected prospective data via their respective pre-hospital medical emergency services. Clinical needs understandably took priority in patients with life-threatening injuries and, thus, serial blood sampling was not always possible, rendering some data sets incomplete. This was frequently the case with patients who were extremely injured and required the largest volume of blood component transfusion.

An important limitation in this body of work was the extreme difficulty in ascertaining the precise timing of haemorrhage control in severely injured and acutely bleeding patients. This was further compounded by the fact that microvascular bleeding may continue after haemorrhage control or the repair of major vascular injury. Throughout this thesis, I have taken a pragmatic approach and used a surrogate marker of the cessation of blood product administration as a possible alternative. Furthermore, to ensure I capture the acute phase of the bleeding response, all of these patients would have had control of haemorrhage within the first one to two hours of their arrival in the emergency department. Clearly, the more shocked a patient is, or the longer they remain shocked, the worse the coagulopathy is likely to be and will further drive the bleeding, shock and coagulopathic process.

While bleeding is important, there is building evidence that suggests that coagulopathy actually represents a perturbed inflammatory milieu and is associated with endothelial, epithelial, and organ dysfunction. Hence, whether haemostatic resuscitation prevents mortality by the attenuation of bleeding or the attenuation of an ‘endotheliopathy’ remains a crucial question. This will only be addressed by a comprehensive characterisation of coagulation and inflammation after injury, coupled with animal and *in vitro* based studies.

I prospectively captured PRBC interval data on coagulation response to reduce the biases present in previous retrospective studies. However, certain temporal limiting effects do remain, and cannot be examined in this study due to the relatively small patient numbers. In a rapidly exsanguinating trauma patient, a lack of immediately available FFP will prohibit the transfusion of higher FFP:PRBC ratios, such that patients who bleed more briskly tend to receive lower ratios as do patients who die of exsanguination (54). When blood component therapy is considered as a time-dependent covariate, the survival advantage becomes less clear. I have shown that patients are more likely to receive higher ratios between PRBC 8 and 12. However, the interval cohorts may be influenced by confounding due to the institution’s transfusion practice, rate of blood loss, or early haemorrhage control. Patients with rapid bleeding are more likely to require 8 U of PRBCs and enter the 8 U to 12 U interval, by which time FFP is available, thereby increasing the FFP:PRBC ratio. Similarly, patients who have brisk haemorrhage control or for whom bleeding stops spontaneously may not require 8 U and, therefore, are unlikely to have received a high dose of plasma as a result of our transfusion protocol. Thus, some patients died not because they did not receive treatment; they did not receive treatment because they died.

In order to reduce survivor bias, I have purposefully excluded mortality data and concentrated on the coagulation profile as a primary outcome. If anything, non-survivors

would possibly have a higher bleeding rate and are likely to have worse coagulation profiles during resuscitation. However, I do not believe their exclusion materially affects any of the results I am presenting, as I am not reporting survival or other outcome effects, only coagulation profile response during haemorrhage. Furthermore, for the overall increasing coagulopathy across groups, ‘survivor bias’ would lead to an underestimation of effect. Patients with a sample at PRBC 12 are alive. Patients at PRBC 8 who then die from haemorrhage would likely have had a worse coagulopathy and, therefore, my results would, if anything, underestimate the trend if this effect were real. In this small study, it is difficult to precisely quantify the influence these factors could have on my data. A future study that incorporates a larger number of patients and that should allow these temporal effects to be fully analysed is currently ongoing.

The confidence intervals were very wide in places, and suggested this investigation lacks a sufficient number of patients to achieve statistical significance. Indeed, although there was a large overall study population, certain categories, such as deceased patients  $\geq 65$  years with an ISS  $< 5$ , have few or no patients; this will have led to incomplete data analyses. As a result of all these, the statistical validity of the results may have been undermined, and thus the strength of the study’s overall conclusions weakened. Furthermore, as a relatively inexperienced researcher at the start of my studies, I acknowledge the limitations of my statistical analysis. Throughout the experimental chapters, I used simple yet appropriate statistical tests to compare pairs or groups. In Chapter Three, I measured the effect of implementing a MHP on clinical outcomes such as length of stay. However, the introduction of a MHP may not be the only factor associated with a change in outcome, and confounders such as age, gender and admission shock could also play a role. While patients analysed in this chapter and in subsequent studies received standardised transfusion protocols, surgical interventions and comparable ICU management, confounders might have been accounted for using multivariate logistic regression modeling. In the future, I will seek further statistical

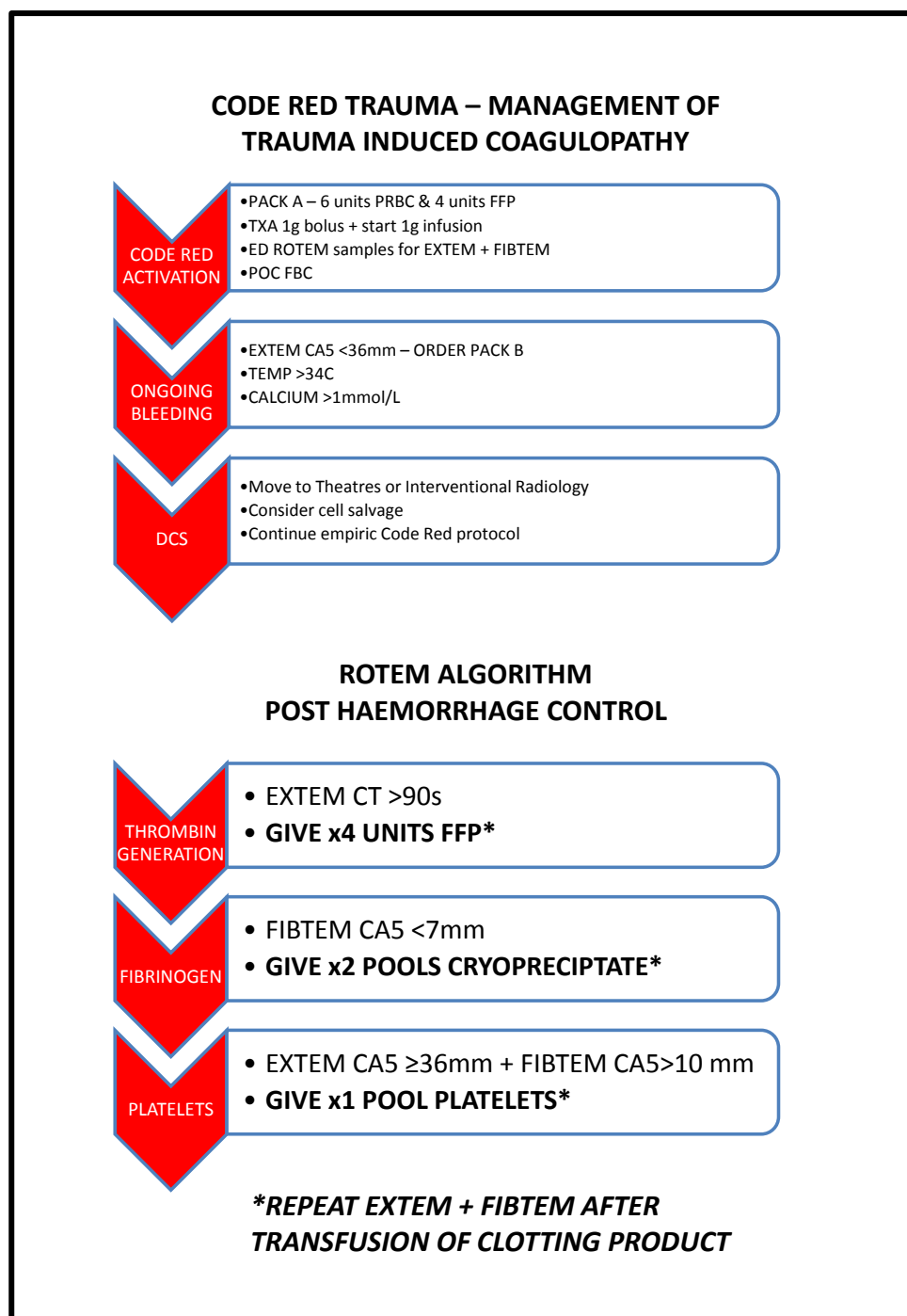


advice that will allow me to account for confounders and identify strong associations between blood component therapy and outcomes.

Finally, many of the conclusions in this thesis are based on associations made in relatively small study populations. Although larger than many previous investigations, the existing data would benefit from substantiation in future studies using greater statistical power.

### **7.3 Clinical importance and future work**

The devastating effect of ATC on the morbidity and mortality of trauma patients underlines both the social and scientific imperative for a better understanding of the treatment of this condition. There is an engaging and important debate about the optimum treatment or prevention strategy required to target ATC. Ultimately, the aim of the research generated and presented in this thesis will be to identify opportunities to reduce the mortality and morbidity associated with trauma. Little was known about the effect of DCR on the coagulation profile during trauma haemorrhage. This work has shown that DCR, in the form of MHPs, is beneficial in the trauma setting but, in average doses, blood component therapy (while maintaining coagulopathy) has little effect on reversing TIC. Of all the blood components, high-dose fibrinogen (in the form of FFP or CRYO) exerted the greatest benefit. The body of knowledge generated by this thesis has directly changed the management of the severely injured and bleeding trauma patient. The new revised Code Red protocol incorporating goal-directed ROTEM parameters at the RLH is detailed in *Figure 7.1*.



**Figure 7.1. The RLH's revised major haemorrhage protocol. (2014)**

Cryoprecipitate is the standard method of fibrinogen supplementation in the UK. Fibrinogen concentrate (FgC) is also available, and is increasingly being used as therapy for major haemorrhages. A Cochrane Review of six small trials, none in a trauma setting, evaluated the effectiveness of FgC for patients with bleeding. It found limited data and reported no effect on overall mortality, but did find a reduction in allogeneic

blood transfusion (203). The evidence relating to the clinical effectiveness of CRYO is more limited, with no randomised controlled trials completed to date (204). Randomised controlled trials evaluating transfusion therapy in trauma are further complicated by virtue of the emergent clinical situation, availability of research personnel outside normal working hours, and time pressure with regard to administering the thawed blood product within a clinically relevant time frame. The body of work within this thesis supports the need for larger studies to determine the clinical benefits of early fibrinogen supplementation.

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## **APPENDICES**

## Appendix 1. ACIT II patient information sheet



### Patient information sheet

DIRECTORATE OF SURGERY AND ANAESTHESIA  
ROYAL LONDON HOSPITAL, WHITECHAPEL, LONDON E1 1BB

#### Information Sheet A: Subject

Version 2, 10.09.2010

#### East London and the City Research Ethics Committee 1

REC number: 07/Q0603/29

#### Title: Activation of Coagulation & Inflammation in Trauma II

**Principal Investigator:** Professor Karim Brohi, FRCS FRCA

Date: \_\_/\_\_/\_\_

Subject Name: \_\_\_\_\_ NHS Ref: \_\_\_\_\_ Study Ref: \_\_\_\_\_

#### Introduction

You are being invited to take part in a research study. This research will help us to improve the care of patients who suffer severe injuries in the future. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

#### Why is this research being carried out?

Trauma (serious injury) is the leading cause of death and disability in children and young adults worldwide. Over half of all trauma deaths are due to bleeding or the complications resulting from it. Injury, shock and blood loss all contribute to a failure of the body's normal blood clotting mechanisms (coagulation), which then leads to more bleeding. The mechanisms of these disorders in blood clotting and how they affect the body are not well understood, and we hope that this research will help us to determine why, when and how the blood clotting mechanisms fail, and what the consequences of this are.

10.09.2010 Info Sheet A – Subject. V2 Page 1

Date: \_\_/\_\_/\_\_ Researcher Initials: \_\_\_\_\_

**Why have I been chosen?**

On \_\_\_\_ - \_\_\_\_ - \_\_\_\_ (date), you were injured and admitted to the Royal London Hospital. At the time, you were unable to give informed consent. When you arrived in the emergency department, a full trauma team of doctors and nurses attended to you. The trauma team leader, who is not part of this research study, gave consent as your representative for you to be enrolled in this study. As part of the immediate management blood is taken and sent to the laboratory for analysis. A small amount of extra blood (approximately six teaspoonfuls) was drawn and saved for research purposes. We are now asking for your consent to allow us to use the samples we have collected and to continue to participate in the study, since all the procedures have not yet been completed. Should you not wish to continue your involvement in the trial we may ask for your consent to place the samples already collected in a registered research tissue bank for use in future research.

**Do I have to take part?**

No, participation is completely voluntary. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

**What will happen to me if I take part?**

If you agree to continue with the study the following will happen:

1. We will store and process the samples we have already collected.
2. We will continue to collect blood samples until the third day in hospital. We will draw \_\_\_\_ blood samples in total. \_\_\_\_ (number) of these have already been obtained. Each blood sample is equivalent to six teaspoonfuls, and the total amount of blood drawn over three days is less than six fluid ounces. Wherever possible we will draw the blood out of a line already in a blood vessel, or coincide the blood draw with tests required for your care, in order to minimize any discomfort from the procedure.
3. At day 28 or on your day of discharge (whichever is sooner) and again at one year following your injury and admission to hospital we will provide you with a short questionnaire to complete. Each questionnaire should take approximately five minutes to complete. The answers to the questions will provide us with valuable information about how you perceive your quality of life after the injury as well how many times you have had to visit healthcare professionals (doctors, physiotherapists, specialist nurses etc). All answers to the questions will remain confidential.

10.09.2010 Info Sheet A – Subject. V2 Page 2

Date: \_\_\_\_ / \_\_\_\_ / \_\_\_\_ Researcher Initials: \_\_\_\_



**What do I have to do?**

If you agree to continue with the study the following will happen:

1. We will collect \_\_\_\_ (number) of further blood samples from you, on \_\_\_\_\_  
(date/times)
2. We will ask you to complete a short questionnaire at day 28 or on your day discharge (whichever is sooner) and again at one year following your injury and admission to hospital.

**What are the possible disadvantages and risks of taking part in the study?**

There are no long-term risks to you from participating in this study. The specific risks associated with each sample are as follows:

- Blood samples - the risks of drawing blood include temporary discomfort from the needle stick and bruising.

**What are the possible benefits of taking part in the study?**

There will be no direct benefit to you from participating in this study, but we hope that the information we get will help to improve the care of trauma patients in the future.

**What if there is a problem?**

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

**Will my taking part in the study be kept confidential?**

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

**This Completes Part I.**

*If the information in Part 1 has satisfied you and you are considering continuing in the study, please read the additional information in Part 2 before making any decision.*

## **PART 2**

### **What will happen if I don't want to carry on with the study?**

If you decide, at any time, to withdraw from the study all study procedures will be stopped immediately. Any information and samples that have already been collected will be processed as part of the study unless you wish to have your samples withdrawn from the study, in which case we will destroy them. Your decision will in no way result in a change in the type or quality of care you subsequently receive. Should you not wish to remain in the trial we may ask for your consent to place the samples already collected in a registered research tissue bank for use in future research.

### **What if I am not happy about the study?**

We will only make very minor changes to the way we look after you. It is extremely unlikely that this small change to normal practice would cause any problems. However, if you are harmed by taking part in this study, there is no special compensation arrangement. If you are harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay your legal costs. Regardless of this, if you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you. Please contact Patient Advisory Liaison Service (PALS) if you have any concerns regarding the care you have received, or as an initial point of contact if you have a complaint. Please telephone 020 3594 2040, or email [pals@bartshealth.nhs.uk](mailto:pals@bartshealth.nhs.uk). You can also visit PALS by asking at any hospital reception.

### **Will my taking part in the study be kept confidential?**

All information which is collected about you during the course of the research will be kept strictly confidential and will be stored securely in coded form. Information about you and your samples will be identifiable only in a coded format, separate from your personal information. Only authorised personnel such as researchers and research auditors will have access to the data.

### **What will happen to your study data and samples?**

Your study data and samples (i.e. materials) will always be collected, stored, transferred and used in a secure and ethical manner that ensures protection of your fundamental rights and privacy. All materials will initially be processed at the research centre where you were enrolled and linked to you by a unique study identifier (i.e. coded). As part of this research study, we would also like to add some of the materials that have been collected to an international trauma database and tissue bank. These centralised collections will be used by trauma investigators across the world, to increase the

**Who has reviewed the study?**

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by East London & City Research Ethics Committee 1.

**Who can I contact for further information?**

1. If you require further information about the study, please contact the ACIT researchers by telephone: 020 3594 0731 or by email: [Claire.rourke@bartshealth.nhs.uk](mailto:Claire.rourke@bartshealth.nhs.uk) or [sirat.khan@qmul.ac.uk](mailto:sirat.khan@qmul.ac.uk)
2. If you require impartial, local advice, please contact the Patient Advice and Liaison Service, telephone: 020 3594 2040 or e-mail: [pals@bartshealth.nhs.uk](mailto:pals@bartshealth.nhs.uk).

**Thank you for taking the time to read this information.**

Date: \_\_/\_\_/\_\_\_\_ Researcher Signature: \_\_\_\_\_

## Appendix 2. Personal Consultee (PC) information sheet



### Personal Consultee (PC) information sheet

DIRECTORATE OF SURGERY AND ANAESTHESIA  
ROYAL LONDON HOSPITAL, WHITECHAPEL, LONDON E1 1BB

#### Information Sheet E: PC

Version 2, 10.09.2010

#### East London and the City Research Ethics Committee 1

REC number: 07/Q0603/29

#### Title: Activation of Coagulation & Inflammation in Trauma II

**Principal Investigator:** Professor Karim Brohi, FRCS FRCA

Date: \_\_/\_\_/\_\_

Subject Name: \_\_\_\_\_ NHS Ref: \_\_\_\_\_ Study Ref: \_\_\_\_\_

#### Introduction

We would like to invite you to agree to your relative/significant other taking part in a research study, while they are unable to give consent for themselves. This research will help us to improve the care of patients who suffer severe injuries in the future. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you agree to your relative/significant other taking part.

#### Why is this research being carried out?

Trauma (serious injury) is the leading cause of death and disability in children and young adults worldwide. Over half of all trauma deaths are due to bleeding or the complications resulting from it. Injury, shock and blood loss all contribute to a failure of the body's normal blood clotting mechanisms (coagulation), which then leads to more bleeding. The mechanisms of these disorders in blood clotting and how they affect the body are not well understood, and we hope that this

research will help us to determine why, when and how the blood clotting mechanisms fail, and what the consequences of this are.

**Why has my relative/significant other been chosen?**

On \_\_\_\_ - \_\_\_\_ - \_\_\_\_ (date), your relative/significant other was injured and admitted to the Royal London Hospital. They were unable to give informed consent. When they arrived in the emergency department, a full trauma team of doctors and nurses attended to them. The trauma team leader, who is not part of this research study, gave consent as a representative for your relative/significant other to be enrolled in this study. As part of the immediate management blood is taken and sent to the laboratory for analysis. A small amount small amount of extra blood (approximately six teaspoonfuls) was drawn and saved for research purposes. We are now asking for your consent to allow us to use the samples we have collected and to allow your relative/significant other to continue in the study, since all the procedures have not yet been completed. Should you not wish your relative/significant other to remain in the trial we may ask for your consent to place the samples already collected in a registered research tissue bank for use in future research.

**Do I have to agree?**

No, participation is completely voluntary. It is up to you to decide whether or not your relative/significant other should take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your relative/significant other receives.

**What will happen to my relative/significant other if they take part?**

If you agree to continue with the study the following will happen:

1. We will store and process the samples we have already collected.
2. At day 28 or on the day of discharge (whichever is sooner) and again at one year following your injury and admission to hospital we will provide your relative/significant other with a short questionnaire to complete. Each questionnaire should take approximately five minutes to complete. If your relative/significant other is unable to complete the questions we may ask you to assist in the process and/or answer some of the questions on their behalf. The answers to the questions will provide us with valuable information about how they perceive their quality of life after the injury as well how many times they have had to visit healthcare professionals (doctors, physiotherapists, specialist nurses etc). All answers to the questions will remain confidential.

We will continue to collect blood samples until the third day in hospital. We will draw \_\_\_\_ blood samples in total. \_\_\_\_ (number) of these have already been obtained. Each blood sample is equivalent to six teaspoonfuls, and the total amount of blood drawn over three days is less than six fluid ounces. Wherever possible we will draw the blood out of a line already in a blood vessel, or coincide the blood draw with tests required for care, in order to minimize any discomfort.

**What do I have to do?**

If you agree for your relative/significant other to continue with the study the following will happen:

1. We will collect \_\_\_\_ (number) of further blood samples on \_\_\_\_\_  
(date/times)
2. We will ask your relative/significant other to complete a short questionnaire at day 28 or on their day discharge (whichever is sooner) and again at one year following their injury and admission to hospital. Completion of the questionnaire may require your assistance if your relative/significant other is unable answer the questions themselves.

**What are the possible disadvantages and risks of taking part in the study?**

There are no long-term risks from participating in this study. The specific risks associated with each sample are as follows:

1. Blood samples

The risks of drawing blood include temporary discomfort from the needle stick and bruising.

**What are the possible benefits of taking part in the study?**

There will be no direct benefit to your relative/significant other from participating in this study, but we hope that the information we get will help to improve the care of trauma patients in the future.

**What if there is a problem?**

Any complaint about the way you or your relative have been dealt with during the study or any possible harm they might suffer will be addressed. The detailed information on this is in Part 2.

**Will taking part in the study be kept confidential?**

Yes. We will follow ethical and legal practice and all information about your relative/significant other will be handled in confidence. The details are included in Part 2.

**This Completes Part 1**

*If the information in Part 1 has satisfied you and you are considering continuing in the study, please read the additional information in Part 2 before making any decision.*

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_ Researcher Initial: \_\_\_\_

**PART 2**

**What will happen if I don't want to carry on with the study?**

If you or your relative/significant other decides, at any time, to withdraw from the study all study procedures will be stopped immediately. Any information and samples that have already been collected will be processed as part of the study unless you wish to have your samples withdrawn from the study, in which case we will destroy them. Your decision will in no way result in a change in the type or quality of care you subsequently receive. Should you not wish for your relative/significant other to remain in the trial we may ask for your consent to place the samples already collected in a registered research tissue bank for use in future research.

**What if I am not happy about the study?**

We will only make very minor changes to the way we look after your relative/significant other. It is extremely unlikely that this small change to normal practice would cause any problems. However, if they are harmed by taking part in this study, there is no special compensation arrangement. If they are harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay your legal costs. Regardless of this, if you wish to complain or have any concerns about any aspect of the way you or your relative have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you. Please contact Patient Advisory Liaison Service (PALS) if you have any concerns regarding the care you have received, or as an initial point of contact if you have a complaint. Please telephone 020 3594 2040, or email [pals@bartshealth.nhs.uk](mailto:pals@bartshealth.nhs.uk). You can also visit PALS by asking at any hospital reception.

**Will my taking part in the study be kept confidential?**

All information which is collected about you during the course of the research will be kept strictly confidential and will be stored securely in coded form. Information about you and your samples will be identifiable only in a coded format, separate from your personal information. Only authorised personnel such as researchers and research auditors will have access to the data.

**What will happen to your study data and samples?**

Your study data and samples (i.e. materials) will always be collected, stored, transferred and used in a secure and ethical manner that ensures protection of your fundamental rights and privacy. All materials will initially be processed at the research centre where you were enrolled and linked to you by a unique study identifier (i.e. coded). As part of this research study, we would also like to add some of the materials that have been collected to an international trauma database and tissue bank. These centralised collections will be used by trauma investigators across the world, to increase the scale and impact of injury research projects. This will reduce the time taken to bring benefit to future trauma patients.

Some materials will be transferred to a central data registry (in the UK) and tissue bank (in Denmark). Materials may then be provided to external researchers for approved injury research.

The design and secure usage of both of these coordinating research facilities is according to national safety and governance standards (e.g. UK Data Protection Act 1998, Directive 95/46/EC, Oviedo Convention Rec (2006)4). Study materials will not be able to be linked back you, other than by responsible personnel at the site of your original enrolment. The coded data and samples will only be accessed by authorised personnel (e.g. research fellows, study auditors).

We will not share your personal details (e.g. name, address). Research conducted may contain key personal information of necessity for research (e.g. age, gender, clinical and treatment data) but all study information will be anonymised at the end of any research project, when results are published and you will not receive the results of any future research project. All future research projects will have been approved by a Research Ethics Committee. We hope that this will allow us to identify new areas of investigation and potentially allow future trauma care to be specifically tailored to the characteristics of each individual patient.

**Will any genetic tests be done?**

Your study data and samples will be used for more than one study, including studies aimed at identifying genetic differences in patients that makes them more or less susceptible to the effects of traumatic injury. We will store a sample of your DNA, obtained from the blood sample for future testing. This is performed to see if there are genetic differences between patients that make them

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Date: \_\_ / \_\_ / \_\_ Researcher Initials: \_\_\_\_



more or less susceptible to the effects of injury. We will not be testing for genetic diseases or named inheritable conditions and therefore this test will be of no individual significance to yourself in terms of inherited risk, insurance issues or to your children.

The DNA will be stored in a coded form in a special DNA bank, with the same data protection safeguards that apply to your other blood samples. Any future studies outside the scope of this study that would use your DNA will have to be independently authorised by a research ethics committee. If you wish not to have your DNA stored, please sign to this effect in the appropriate part of the consent form.

**What will happen to the results of the research study?**

We hope to publish the results in a scientific journal. It will not be possible to identify any individual who has taken part from this scientific report. Copies of the report will be available on request.

**Who has reviewed the study?**

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by East London & City Research Ethics Committee 1.


**Who can I contact for further information?**

1. If you require further information about the study, please contact the ACIT researchers by telephone: 020 3594 0731 or by email: [Claire.rourke@bartshealth.nhs.uk](mailto:Claire.rourke@bartshealth.nhs.uk) or [sirat.khan@qmul.ac.uk](mailto:sirat.khan@qmul.ac.uk)
2. If you require impartial, local advice, please contact the Patient Advice and Liaison Service, telephone: 020 3594 2040 or e-mail: [pals@bartshealth.nhs.uk](mailto:pals@bartshealth.nhs.uk)

**Thank you for taking the time to read this information.**

Date: \_\_/\_\_/\_\_\_\_ Researcher Signature: \_\_\_\_\_

## Appendix 3. Subject consent form

		
<b>Consent form</b> DIRECTORATE OF SURGERY AND ANAESTHESIA ROYAL LONDON HOSPITAL, WHITECHAPEL, LONDON E1 1BB		
<b>Consent Form A – Subject</b> Version 2, 10.09.2010		
<b>East London and the City Research Ethics Committee 1</b>	REC number: 07/Q0603/29	
<b>Title: Activation of Coagulation &amp; Inflammation in Trauma</b>		
<b>Principal Investigator:</b> Professor Karim Brohi, FRCS FRCA	Please initial box to Indicate agreement	
1. I confirm that I have read and understood the information sheet dated 10.09.2013 (version 3.2) for the above study and have had the opportunity to ask questions. I have been given a copy of the patient's information sheet to keep.	[      ]	
2. I understand that my participation in this study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	[      ]	
3. I understand that sections of any of my medical notes may be looked at by professional individuals involved in this study or by regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.	[      ]	
4. I understand that I have given my consent voluntarily to the international transfer, storage and use of my study data and tissue for future medical research and that I am free to withdraw my consent at any time.	[      ]	
5. I agree that the tissue may be used for future genetic research but not for research that involves reproductive cloning or testing for inherited diseases without my express consent.	[      ]	
6. I agree to take part in the above study.	[      ]	
_____ Name of patient	_____ Date	_____ Signature
I have explained this in terms which, in my judgement, are suited to the understanding of the patient.		
_____ Name of person taking consent (if different from Investigator)	_____ Date	_____ Signature
_____ Investigator	_____ Date	_____ Signature
Consent Form A – Subject. V3.2	1	10.09.2010

**Consultee declaration form**

DIRECTORATE OF SURGERY AND ANAESTHESIA  
ROYAL LONDON HOSPITAL, WHITECHAPEL, LONDON E1 1BB

**Consent Form B – Personal Consultee (PC) Declaration Form**

Version 2.0, 10.12.2010

**East London and the City Research Ethics Committee 1**

REC number: 07/Q0603/29

**Title: Activation of Coagulation & Inflammation in Trauma**

**Principal Investigator:** Professor Karim Brohi, FRCS FRCA

Please initial box to  
Indicate agreement

1. I confirm that I have read and understood the information sheet dated 10.09.2013 (version 3.2) for the above study and have had the opportunity to ask questions. I have been given a copy of the patient's information sheet to keep. [      ]
2. In my opinion the patient would have no objection to taking part in the above study [      ]
3. I understand that I can request the patient's withdrawal from the study at any time, without giving any reason and without the patient's medical care or legal rights being affected. [      ]
4. I understand that relevant sections of the patient's medical notes and data may be looked at by responsible individuals involved in this study or by regulatory authorities where it is relevant to their taking part in research. [      ]
5. In my opinion, the patient would have no objection to the international transfer, storage and use of their study data and tissue for future medical research. [      ]
6. I agree that the patient's tissue may be used for future genetic research but not for research that involves reproductive cloning or testing for inherited diseases without express consent. [      ]
7. I agree to allow my relative/significant other to take part in the above study. [      ]

\_\_\_\_\_  
Name of Consultee                      Date                      Signature

I have explained this in terms which, in my judgement, are suited to the understanding of the Consultee.

\_\_\_\_\_  
Person undertaking consultation (if different from Investigator)                      Date                      Signature

\_\_\_\_\_  
Investigator                      Date                      Signature

Consent Form B - PC. V2

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10.12.2010

## Appendix 4. Consultee declaration form



## Appendix 5. Consent to donation and storage of blood samples

Barts and The London   
NHS Trust



### CONSENT TO DONATION AND STORAGE OF RESIDUAL TISSUE FOR MEDICAL RESEARCH

Study Title: **Activation of Coagulation & Inflammation in Trauma II**

#### Research Ethics Committee Reference:

Following the present research project any residual (left-over) tissue, may be collected, stored and used by the Barts and the London NHS Trust and / or Queen Mary's School of Medicine and Dentistry, for medical research in the future. Research conducted on these samples may contain personal information but all such information will be anonymised at the end of any project, when the results are published, and you will not receive the results of any future research project. All staff undertaking future studies will abide by the Data Protection Act 1998 with any medical information relating to you being kept confidential. The tissue may be given to external research organisations for approved medical research but tissue will not be sold, although costs will be recovered without any financial benefit to either you or to the researcher. Any residual tissue will be disposed of lawfully when it is no longer required.

Patient Initials

I understand that I have given my consent voluntarily to the storage of tissue for future medical research and that I am free to withdraw my consent at any time ☐

I agree that the tissue may be used for future genetic research but not for research that involves reproductive cloning, or be tested for inherited diseases without my express consent ☐

If you have any preferences or exclusions for use of the donated tissue, or any other comments, please include them here:

\_\_\_\_\_  
Name of Patient

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Name of Person taking consent  
(Researcher)

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

1 copy for Patient, 1 for medical notes  
original to be kept in research file and sent with any sample to the HTRC when applicable

Consent Form C – Tissue. v1.3

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20.09.2007